ADRENAL CORTEX

Transactions of the Third Conference
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JOSIAH MACY, JR FOUNDATION CONFERENCE PROGRAM

As an introduction to these Transactions of the Third Conference on Adrenal Cortex I should like to outline what it is that the Foundation hopes to accomplish by its Conference Program

We are interested first of all in furthering knowledge about adrenal cortex and to this end the participants were brought together to exchange ideas experiences data and methods In addition to this particular goal however there is a further and perhaps more fundamental aim which is shared by all our conference groups the promotion of meaningful communication between scientific disciplines

The problem of communication between disciplines we feel to be a very real and very urgent one the most effective advancement of the whole of science being to a large extent dependent upon it Because of the accelerating rate at which new knowledge is accumulating and because discoveries in one field so often result from information gained in quite another channels must be established for the most relevant dissemination of this knowledge

The increasing realization that nature itself recognizes no boundaries makes it evident also that the continued isolation of the several branches of science is a serious obstacle to scientific progress Particularly is it so in medicine that the limited view through the lens of one discipline is no longer enough For example today medicine must be well versed in nuclear physics because of the tracer techniques and the injury which can result from radiation At the other extreme medicine is certainly a social science and through mental health must be concerned with economic and social questions The answer then is not further fragmentation into increasingly isolated specialities disciplines and departments but the integration of science and scientific knowledge for the enrichment of all branches This integration we feel can be encouraged by providing opportunities for a multiprofessional approach to given topics

Although the fertility of the multidiscipline approach is recognized adequate provision is not made for it by our universities scientific societies and journals And perhaps the presence of other hindering factors must be admitted Partly semantic in nature they may also to some degree be psychological Admittedly it is oftentimes difficult to accept data derived from methods with which one is unfamiliar By

making free and informal discussion the central core of our meetings we hope to achieve an atmosphere which minimizes as much as possible these emotional barriers

Thus our meetings are in contrast to the usual scientific gatherings They are not designed to present neat solutions to tidy problems but to elicit provocative discussion of the difficulties which are being encountered in research and practice For this reason we ask that the presentations be relatively brief and that emphasis be placed on discussion as the heart of the meeting Our hope is that the participants will come prepared not to defend a single point of view but to take advantage of the meeting as an opportunity to speak with representatives of other disciplines in much the same way that they would talk with their own colleagues in their own laboratories

We have now thirteen groups functioning under the Conference Program on the following topics adrenal cortex aging blood clotting connective tissues consciousness cybernetics infancy and childhood liver injury metabolic interrelations nerve impulse renal function cold injury and shock and circulatory homeostasis

When a new conference is organized the Chairman in consultation with the Foundation selects fifteen scientists to be the nucleus of the group and every effort is made to include representatives from all pertinent disciplines From time to time new members are added by the group to fill gaps in viewpoint or technique A limited number of guests are invited to attend each meeting, but for the purpose of promoting full participation by all members and guests attendance at any meeting is limited to twenty five It is inevitable that in no topic can we possibly include more than a small fraction of the key investigators in the field and one of the difficulties in forming a group like this is that it is necessary to leave out so many people whom we would like to include

The transactions of these meetings are recorded and published This is done because the Foundation wishes to make current thinking in a field available to all those working in it and because it believes that conveying to those in other fields who are concerned with science for example government officials administrators etc the essential nature of scientific research is also an important problem in communication Logic is a vital aspect of science but equally essential is the intuitive or creative aspect Research is as creative as the painting of a portrait or the composing of a symphony Although logic is of course necessary in order to rearrange to test and to validate research thrives on creativity which has its source in unconscious nonrational processes

Unfortunately however in the finished products which are presented to the world through research reports this integral part of scientific endeavor is shriveled by the cold white light of logic By preserving the informality of our conferences in the published transactions we hope to give a truer picture of what actually goes on in the minds of scientists and of the role which creativity plays

FRANK FREMONT SMITH M D
Medical Director

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EFFECTS OF ADRENAL CORTICAL HORMONES ON RENAL FUNCTION*

ROBERT F PITTS
Department of Physiology
Cornell University Medical College

IN THE PROCEEDINGS of the 1951 Laurentian Conference Dr Gaunt (1) made the apt observation that in assembling the facts concerning the actions of adrenal cortical hormones on renal function one would have to rely more on clairvoyance than on clarity if one were to produce an integrated story Although the facts which I shall present are largely derived from work of my associates Dr Sartorius Dr Roemmelt Dr Roberts Dr Axelrod and Dr Thompson any spirits which I may conjure up from these facts are strictly my own responsibility

I should like to introduce my remarks with two deceptively simple propositions Although you may agree with them at first you may heartily disagree with the way in which I develop them First no single discrete renal function present in the normal animal is absent or lost in the adrenalectomized animal or in the patient with Addison s disease The adrenal cortical hormones merely modify rates of certain processes which proceed whether the hormones are present or not Second adrenal hormones probably control the rates of several renal processes directly but they modify the rates of many more indirectly Therefore in a discussion of the effects of adrenal cortical hormones on renal function we should attempt to distinguish between those effects which are primary and dependent on a direct regulation of discrete functions and those which are secondary to salt and water retention or to alterations in the composition of the body fluids

Let me illustrate this latter point with a consideration of the problem of adrenal cortical control of glomerular filtration rate and renal plasma flow Talbott *et al* (2) and Waterhouse and Keutmann (3) have observed that glomerular filtration rate and renal plasma flow are consistently reduced in patients with Addison s disease even those presumably adequately sustained with salt and desoxycorticosterone Does

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The author is indebted to The American Heart Association the National Heart Institute of the National Institute of Health and the Life Insurance Medical Research Fund for support of the work presented in this paper which has originated in his laboratory

TABLE II

Immediate Effects of Adrenal Cortical Extract and Desoxycorticosterone on Renal Function of Adrenalectomized Dog

Urine Flow	Glom Fil Rate	Renal Plasma Flow	Plasma Na	Plasma K	Urine Flow	Glom Filt Rate	Renal Plasma Flow	Plasma Na	Plasma K
ml /min			mEq /L		ml /min			mEq /L	
1 35	27 9	91 6	146 5	5 90	0 70	29 2	91 5	143 5	5 94
1 40	26 1	85 8	146 1	5 93	0 70	29 1	87 8	14[illegible] 2	5 86
1 50	24 3	80 6	143 5	5 74	0 60	28 6	91 1	143 2	5 51
1 40	25 8	95 0	145 [illegible]	5 6[illegible]	0 55	32 3	95 7	140 8	5 55
20 ml adrenal cortical extract i v					5 mg DCA i v				
0 61	[illegible]7 7	[illegible]1 0	145 0	5 26	0 60	33 2	102 0	143 2	5 21
0 48	25 5	94 8	142 9	4 95	0 58	31 8	98 0	141 1	5 12
0 [illegible]0	[illegible]8 8	96 0	143 0	5 16	0 35	31 5	105 6	141 3	[illegible] 79
0 65	27 4	98 8	144 5	4 85	0 33	35 1	123 3	141 3	4 75
1 27	30 3	112 0	144 7	4 92	0 45	35 9	137 5	141 3	[illegible] 47
1 50	32 4	122 6	145 2	4 66	0 35	35 0	121 0	141 3	4 66

Reprinted by permission from Roemmelt J C Sartorius O W and Pitts R F Excretion and reabsorption of sodium and water in adrenalectomized dog *Am J Physiol* 159 1[illegible]4 (1949)

TABLE I

Rates of Glomerular Filtration of Five Dogs Immediately Before and from Eight to Ten Days After, Adrenalectomy

Dog No	Control	Adrenalectomized	Days Postoperative
1	61.8	61.9	8
2	34.6	31.2	8
3	53.9	48.7	10
4	63.3	59.7	9
5	57.6	61.0	8

Glomerular filtration rate ml per min

Reprinted by permission from Roemmelt J C Sartorius O W and Pitts R F Excretion and reabsorption of sodium and water in adrenalectomized dog *Am J Physiol* 159 124 (1949)

this mean that some specific cortical hormone is necessary for maintenance of the patency of the renal vascular bed? I doubt it

Roemmelt *et al* (4) studying glomerular filtration rate in five dogs prior to and following bilateral adrenalectomy observed no decrease eight to ten days postoperatively when the animals were maintained in good condition with saline These observations by Roemmelt are summarized in Table I Similar results were obtained in rats by Lotspeich (5) and by Gaunt and others (6) On the other hand Harrison and Darrow (7) have observed reduced glomerular filtration rate and renal plasma flow in untreated animals in acute adrenal insufficiency and have noted that these are restored to normal values by the administration of desoxycorticosterone and adrenal cortical hormone

Do cortical hormones increase glomerular filtration rate and renal plasma flow under conditions of adrenal insufficiency through some primary action on the renal vascular bed or do they exert their effects by maintaining normal extracellular and plasma volumes? In Table II are summarized two experiments on one adrenalectomized dog This animal was maintained on saline and although the plasma sodium concentration was normal the elevated potassium level is an indication of moderate inadequacy of therapy In the first experiment the animal was given 20 ml intravenously of whole adrenal cortical extract and in the second experiment 5 mg of desoxycorticosterone acetate emulsified in saline True enough filtration rate and renal plasma flow increased somewhat following the administration of either hormone

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ml/min			mEq/L		ml/min			mEq/L	
1.35	27.9	91.6	116.5	5.90	0.70	29.2	91.5	113.5	5.94
1.40	26.1	95.8	116.1	5.83	0.70	29.1	97.9	112.2	5.56
1.50	21.3	80.6	113.5	5.71	0.60	8.6	81.1	113.2	5.51
1.10	25.9	85.0	145.[illegible]	5.62	0.55	32.3	95.7	110.8	5.55
20 ml adrenal cortical extract iv					5 mg DCA iv				
0.64	27.7	91.0	115.0	5.26	0.60	33.2	102.0	113.2	5.21
0.48	25.5	81.8	112.9	4.95	0.58	31.8	98.0	111.1	5.12
0.10	29.8	96.0	113.0	5.16	0.35	31.5	105.6	111.3	4.78
0.65	27.4	99.8	114.5	4.85	0.33	35.1	123.3	111.3	4.75
1.27	30.3	112.0	114.7	4.92	0.45	35.9	132.5	111.3	4.17
1.50	32.4	122.6	115.2	4.66	0.55	35.0	121.0	111.3	4.66

Reprinted by permission from Roemmelt, J. C., Sartorius, O. W., and Pitts, R. F.: Excretion and reabsorption of sodium and water in adrenalectomized dog. *Am. J. Physiol. 159: 1[illegible]4 (1949)*

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Dog No	Control	Adrenalectomized	Days Postoperative
1	61 8	61 9	8
2	34 6	31 2	8
3	53 9	48 7	10
4	63 3	59 7	8
5	57 6	61 0	8

Glomerular filtration rate ml per min

Reprinted by permission from Roemmelt J C Sartorius O W and Pitts R F Excretion and reabsorption of sodium and water in adrenalectomized dog *Am J Physiol* 159 *124* *(1949)*

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The drop in glomerular filtration rate in developing adrenal insufficiency may or may not be an active renal compensation for salt and water loss In any event however it is a fortunate occurrence for it limits that loss and hence prolongs life The basis for this statement is evident in an experiment of Dr Thompson s (9) illustrated in Figure 1 This experiment was performed on an adrenalectomized dog Because the experiment was a fairly rigorous one the animal was maintained on minimal doses of hormone until the day before the experiment and then was infused with saline throughout its course a fact which accounts for the excretion of some 900 microequivalents of sodium per minute A balloon catheter positioned in the aorta above the renal arteries permitted control of the renal arterial perfusion pressure and hence of glomerular filtration rate Each time the renal arterial pressure was decreased by inflating the balloon filtration rate dropped and with it urine flow and sodium excretion In the final

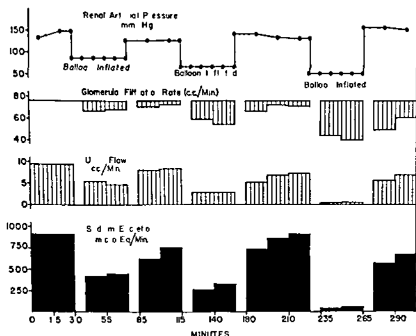


FIGURE 1 Diminished salt and water excretion associated with reduced glomerular filtration rate in an adrenalectomized dog Filtration rate was lowered by inflating a balloon catheter positioned in the aorta above the renal arteries Reprinted by permission from Thompson D D and Pitts R F Effects of alterations of renal arterial pressure on sodium and water excretion *Am J Physiol* 168 *490 (1952)*

but the increase was not especially impressive and certainly did not approach the restoration of control levels of about 60 ml and 180 ml per minute for these two variables Often no change at all was seen in either variable and never even with large doses of cortisone or desoxy corticosterone is glomerular filtration rate or renal plasma flow re turned acutely to anywhere near a normal value in an insufficient animal

Four experiments of Dr Roberts (8) on one adrenalectomized dog performed over a period of five months are summarized in Table III In the initial experiment done about seven weeks after adrenalectomy the animal was in frank adrenal insufficiency with low plasma sodium and high plasma potassium concentrations Glomerular filtration rate and renal plasma flow were both abnormally low Subsequently when maintained on a variety of therapies for periods of ten days to two weeks before the experiments both glomerular filtration rate and renal plasma flow were found to be within the range of normal for this animal Plasma levels of sodium and postassium were essentially nor mal The animal was maintained in a healthy and active condition We assume although we did not measure them that extracellular and plasma volumes were also normal under these conditions In essence we believe that the major effects of cortisone adrenal cortical extract and desoxycorticosterone on glomerular filtration rate and renal plasma flow are not primary but are secondary to their effects on volume and composition of the body fluids If volume and composition are normal it makes no difference whether hormones are present or absent

TABLE III

Plasma Composition and Renal Function in an Adrenalectomized Dog Maintained on a Variety of Therapeutic Regimens

Date	Daily Therapy	Plasma		Glom Filt Rate	Renal Plasma Flow
		Na	K		
	mg	mEq /L		ml /min	
3/21/51	0	132	6 5	30	116
4/13/51	Cortisone (10) DCA (1)	149	4 5	60	184
7/13/51	Cortisone (10) DCA (1)	150	3 5	62	260
8/8/51	Cortisone (25)	148	2 7	67	281

Reprinted by permission from Roberts K and Pitts R F The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog *Endocrinology* 50 51 (1952)

periods the animal excreted about 80 microequivalents of sodium per minute The sodium lost in the urine amounted to only 2 per cent of that filtered through the glomeruli Reabsorption was 98 per cent complete This 2 per cent loss however if continued would lead to the excretion each day of the quantity of sodium contained in 800 ml of extracellular fluid The administration of 20 ml of aqueous adrenal cortical extract intravenously cut the sodium loss nearly to zero within about sixty minutes Absorption rose to become 99 8 per cent complete This increase in absorption amounted to only 2 per cent yet it is a highly significant increase If the animal were kept on a low salt intake it would spell the difference between salt loss and salt balance It is indeed this small continued salt loss which results in the depletion of extracellular sodium reserves and shrinkage of plasma and interstitial fluid volumes in the untreated adrenalectomized animal kept on a low salt intake

Actually one does not know where adrenal steroids act to enhance salt absorption but certainly the most reasonable guess is that they act on the distal segment of the renal tubule It is now thought that 80

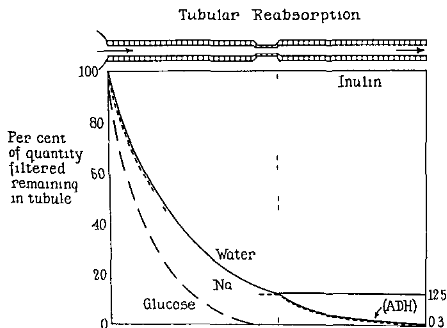


FIGURE 5 Diagrammatic representation of the sites and amounts of reabsorption of certain urinary constituents The author is indebted to Dr Henry Lauson for the use of this Figure

period of balloon inflation during which filtration rate dropped to about half its control value sodium loss was practically abolished A 50 per cent drop in glomerular filtration rate is commonly observed in developing adrenal insufficiency We infer that this drop reduces sodium and water loss and thus prolongs life This constitutes the basis for my statement that whether or not the drop in glomerular filtration rate is an active renal compensation it is a fortunate occurrence

Renal salt loss as a cause of low plasma sodium concentration in patients with Addison s disease and in adrenalectomized animals and the salutary effects of sodium chloride on the symptoms and signs of adrenal insufficiency were first recognized by Loeb (10) and by Harrop (11) and their associates Inadequacy of renal tubular absorption of salt is probably the most clear cut renal dysfunction in adrenal insufficiency It is no doubt a primary one The extent of the dysfunction however is quantitatively very small even though of great significance functionally Figure 2 illustrates how very small is this inadequacy of sodium absorbing capacity This experiment was performed on an adrenalectomized animal maintained on saline *ad lib* for three days after discontinuation of desoxycorticosterone In the three control

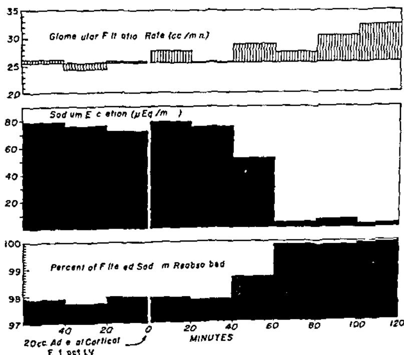


FIGURE 2 Effects of intravenous adrenal cortical extract on the absorption and excretion of sodium in the adrenalectomized dog

TABLE IV

The Immediate Effects of Cortisone on the Renal Tubular Absorption and Excretion of Sodium and Potassium

Time	Urine Flow	Glom Filt Rate	Renal Plasma Flow	Sodium					Potassium	
				Plasma	Urine	Filt	Excr	Reab	Plasma	Excr
mins	ml /min			mEq /L		mEq /min		per cent	mEq /L	μEq /min
Control	0 70	65 5	244	135 5	184	8 43	0 129	98 5	19	81 2
0	Cortisone 20 mg i v									
30	0 58	54 6	240	136 3	96 3	7 09	0 050	99 3	4 5	64 6
60	0 40	60 9	302	135 5	59 9	7 83	0 021	99 7	4 0	11 5
90	0 30	62 3	264	133 2	35 7	7 89	0 011	99 9	3 5	33 1
120	0 23	58 7	214	131 4	22 6	7 33	0 005	99 9	3 5	28 1

Repr ted by pe m n fro i Rob rt K d Pitt R F The nfluence of co t o e on re al funct on and electrolyte excret on the ad e alectomi ed dog *En locri ology* 50 51 (1952)

to 90 per cent of the sodium all of the glucose and most of the other valuable constituents of the glomerular filtrate are actively absorbed in the proximal segment of the renal tubule As these osmotically active constituents are removed from the tubular fluid an osmotic force develops which returns water to the blood stream The absorption therefore of some 80 to 90 per cent of the filtered water is presumed to occur in the proximal segment as a secondary result of the absorption of solutes There is no reason to believe that water is actively absorbed in this segment Indeed as Walker Bott Oliver and MacDowell (12) have pointed out the tubular contents remain isotonic with the plasma throughout the entire length of the proximal segment

Perhaps as Homer Smith (13) claims and as is illustrated in Figure 3 as little as 12 5 per cent of the filtered solutes and water enter the distal tubule Normally only 0 3 to 0 5 per cent of the filtered salt and water are excreted Probably most of the sodium and chloride entering the distal segment are absorbed even in the absence of adrenal hormones for even in marked adrenal insufficiency the fraction of the filtered salt and water excreted is very small Nevertheless it is reasonable to assume that adrenal hormones stimulate the absorption of the final 2 per cent or so of the filtered ions upon which maintenance of balance depends The minute fraction of ion absorption controlled by adrenal hormones makes this moiety extremely difficult to study

In an experiment of Dr Roberts illustrated in Table IV sodium absorption was 98 5 per cent complete in the slightly insufficient adrenalectomized dog Following a relatively large dose of cortisone intravenously absorption increased to become 99 9 per cent complete without significant change in glomerular filtration rate or renal plasma flow These data illustrate again a primary effect of adrenal hormones on the capacity of the renal tubular cells to absorb sodium They likewise illustrate how small a moiety of absorption is involved

There is located in the distal tubule a base-conserving mechanism which in man normally exchanges some 50 to 100 milliequivalents of hydrogen ion and ammonia each day for sodium (14 15) Present concepts as to the nature of this mechanism are illustrated in Figure 4 in a much simplified and diagrammatic fashion As shown by Loeb (16) in severe diabetic ketosis this mechanism can increase its rate of exchange some ten times or more This exchange of hydrogen ions and ammonia for sodium serves to restore base to the body as bicarbonate to replenish reserves depleted in neutralizing fixed metabolic acids Recently Berliner has shown that potassium secretion represents an exchange of potassium ions for sodium ions presumably by this same distal tubular mechanism

Could it be that the small deficiency in sodium absorption which un

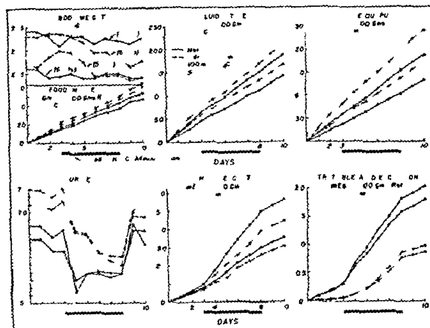


FIGURE 5 Comparison of the renal responses of normal and adrenalectomized rats to the administration of 50 and 100 mg NH_4Cl per day for five days Rats were allowed free access to saline

(Figure 5) by the experiments of Sartorius and myself This chart compares the responses of normal and adrenalectomized rats to acid loads of 50 to 100 mgs of ammonium chloride per day The solid lines describe the behavior of the normal animals the dash lines that of the adrenalectomized animals The large dose of ammonium chloride is represented by the solid symbols the smaller by the open circles All variables except pH are plotted cumulatively The rats were fed *ad lib* and were given free choice of saline or tap water for drinking All animals survived the acid load in good shape although the adrenalectomized animals lost more weight despite the fact that they ate and drank more than did the normal animals

Characteristically adrenalectomized animals excrete urine of higher pH than do normal animals During acid loading the pH of the urine dropped in both groups but very much more slowly in the adrenalectomized animals Accordingly titratable acid excretion increased more in the normal than in the adrenalectomized animals although on the last day the rates of the two groups were nearly the same Ammonia excretion likewise increased more in the normal than in the adrenalectomized animals at each dose level of ammonium chloride It is ap

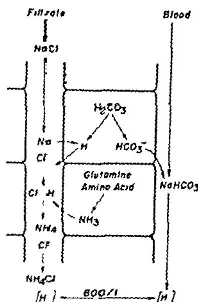


FIGURE 4 Diagrammatic representation of distal tubular processes involved in acid base regulation The primary event is an exchange of hydrogen ions dissociated from carbonic acid within the tubular cells for base in the tubular urine Since the capacity of the tubular cells to produce free acid in this way is limited to the development of a concentration gradient for hydrogen ions of about 800 to 1 the diffusion of ammonia into the urine neutralizing those hydrogen ions is important in allowing continued exchange to occur

derlies the sodium loss of adrenal insufficiency represents inadequacy of this exchange mechanism? Such a view has been expressed by Jiminez Diaz (17) If this concept were true one would expect to find in the adrenal insufficient state reduced body sodium stores reduction of the alkaline reserve that is acidosis diminished excretion of titratable acid and ammonia and finally retention of potassium If one were to whip up the activities of this exchange mechanism by overtreatment with adrenal hormones or by overproduction of these hormones as in Cushing's syndrome one would expect to find increased body sodium stores increased alkaline reserves that is alkalosis increased excretion of titratable acid and ammonia and depletion of body potassium stores The hypothesis is a reasonable one for grossly the theory fits the facts However I might add that the hypothesis is not adequate to account for all sodium loss nor does it account for the well recognized chloride loss in adrenal insufficiency

Adrenalectomy reduces the capacity of the kidney of the rat to exchange hydrogen ion and ammonia for sodium ions as shown

received as ammonium chloride plus an additional 2 milliequivalents but they lost in all only 0 3 of a milliequivalent of sodium in the process The 6 milliequivalents of chloride plus other metabolically produced acids were covered by 1 milliequivalent of hydrogen ion 5 5 milliequivalents of ammonia and 2 5 milliequivalents of potassium The normal animals lost weight but suffered no overwhelming acidosis

In contrast the adrenalectomized rats excreted only 2 out of the 5 milliequivalents of chloride they received in the three days in which they survived the experiment Sodium loss was some three times as great as that of the normal animals and a much smaller than normal proportion of acid anion was eliminated combined with potassium ammonia and hydrogen ion Weight loss in the adrenalectomized animals which in fair part must represent loss of body fluids was not enough greater than in the normal animals to account for their 100 per cent mortality It is probable that overwhelming acidosis was the significant element and was due to replacement of bicarbonate of the body fluids with chloride and probably with other acid anions as well

There is one serious and obvious difficulty with these experiments Adrenalectomized rats placed on a low salt intake and loaded with ammonium chloride do not eat any appreciable quantity of food Accordingly one must immediately discount the reduced excretion of potassium in the adrenalectomized group They simply did not take in as much potassium as did the normal animals This difference in the food intake of the adrenalectomized and normal animal exposed to stress illustrates rather well the difficulties that one runs into in balance experiments In fact it is fairly evident that we are not as we desired to do studying the inherent capacity of the kidney to exchange hydrogen ions and potassium ions or ammonium ions for sodium Rather we are studying that capacity as secondarily modified by inadequate food intake There is little doubt but what these adrenalectomized animals were suffering from a starvation type of ketosis

Fremont Smith Does the falling off in fluid intake in the adrenalectomized animal introduce another factor also?

Pitts Yes that is another complicating factor in this experiment The adrenalectomized rats did not ingest as much water as did the normal animals

Fremont Smith What would have happened if you had given them that extra water by stomach tube so that you would have been dealing with the same amounts of fluid available and the experiments would have been more comparable?

Pitts I quite agree with you that the experiment should have been done another way As a Monday morning quarterback I would say that we should have force fed and forced fluids in the adrenalectomized

parent from these experiments that the adrenalectomized animal responds much less adequately to an acid load and certainly much more slowly than does the normal animal Nevertheless the adrenalectomized animal does retain an inherent capacity to regulate its acid base balance a fact which illustrates one of my initial points namely that the adrenal cortical hormones modify rates of certain renal processes which proceed whether the hormones are present or not

A fact of very great significance which permitted the survival of an acid load by the adrenalectomized animals is that they were given free access to saline When the rats were placed on a salt free diet and were given 100 mgs of ammonium chloride per day none survived four days of acid loading The results of these experiments are summarized in Figure 6 all rats received approximately 1 milliequivalent of chloride per day per 100 grams body weight as ammonium chloride The normal rats excreted in four days the 4 milliequivalents of chloride they had

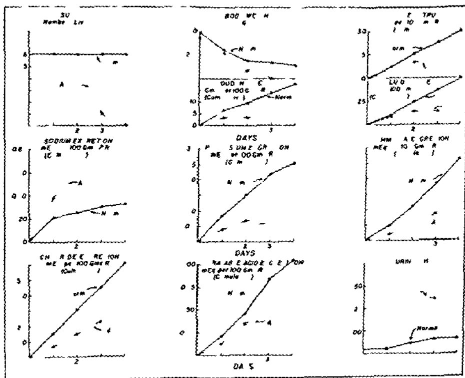


FIGURE 6 Comparison of the renal responses of normal and adrenalectomized rats to the administration of 100 mg NH_4Cl per day for the duration of the experiment Rats were maintained on a salt free diet

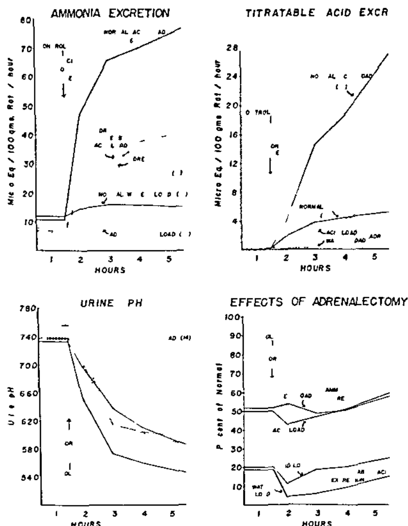


FIGURE 7 Comparison of the immediate renal responses of normal and adrenalectomized rats to a severe acid load imposed by the administration of a single dose of 100 mg of NH_4Cl dissolved in 2 ml of water per 100 gms body weight and to a very mild dilution acidosis induced by the administration of 2 ml of water alone per 100 gms body weight Two groups of adrenalectomized rats were studied one operated two weeks the other operated two months prior to the experiment

animals Even better we should have paired the food and fluid intakes of the normal and adrenalectomized groups However we accomplished the same end in another way We switched over to doing acute experiments We started with adrenalectomized animals maintained on saline that were in good shape We thus avoided variations in the state of health food and fluid intake of the control and adrenalectomized groups

In Figure 7 are compared the responses to two types of acid stress of normal animals and animals adrenalectomized either two weeks or two months The first and very mild acid stress is the dilution acidosis which results when a load of 2 ml of distilled water per 100 grams of body weight is given by mouth The second and much more severe acid stress is that of administering 100 milligrams of ammonium chloride per 100 grams of body weight dissolved in this same amount of water namely 2 ml per 100 grams

Normal animals *eliminating some 11 to 12 microequivalents of am*monium per hour during the control period increased their output to 17 to 18 microequivalents in the dilution acidosis following water and to 75 microequivalents in a more severe acidosis induced by the administration of ammonium chloride Adrenalectomized animals excreting am*monia at about one half the control rate of the normals increased their* output after water and after ammonium chloride by about one half the normal amount In fact as is evident in the lower right hand segment of Figure 7 the output of ammonia by the adrenalectomized animal is about 50 per cent of that of the normal animal during the control peri*ods following water loading and following ammonium chloride load*ing The deficit in the capacity of the adrenalectomized animal to eliminate titratable acid was even more marked than was the deficit in ammonium production In fact the water loaded normal animal put out more titratable acid during the first three hours of the experiment than *did the acid loaded adrenalectomized animal It is apparent that under* all conditions the adrenalectomized animal has only 10 to 20 per cent of the capacity of the normal animal to eliminate titratable acid Figure 8 illustrates the fact that desoxycorticosterone very significantly increases the capacity of the adrenalectomized animal to excrete ammonia *and titratable acid A comparison of Figures 7 and 8 illustrates in addi*tion the fact that the restoration of these two renal functions in the adrenalectomized animal by desoxycorticosterone is essentially complete In these experiments one half milligram of desoxycorticosterone was administered eighteen hours and one hour prior to the start of the experiment *It is evident* that the administration of desoxycorticosterone raised the output of ammonia and titratable acid during the control the dilution acidosis and the ammonium chloride acidosis periods to normal

or essentially normal levels In other experiments not illustrated here it was observed that whole adrenal cortical extract administered in a dose of 1 ml eighteen hours and one hour prior to an experiment like wise appeared to restore both ammonia and acid output These experiments on the rat are at least in qualitative agreement with some which were reported in a preliminary fashion by Dr White (18) at the meeting of the Macy Conference Group on Renal Function a year ago Dr White s observations on the adrenalectomized dog indicate a somewhat similar deficit in capacity to excrete titratable acid and ammonia

Ralli May I ask two questions? How long after adrenalectomy were the tests done and did DCA improve the capacity of the rats to excrete the ingested water?

Pitts Two groups of animals were studied namely those adrenalectomized two weeks and those adrenalectomized two months prior to the experiment We were not concerned in either series of experiments with the amount of water excreted by these animals following a water load of 2 ml per 100 grams body weight The capacity of the animals to put out water is still reduced after desoxycorticosterone In other words the water diuresis has not been restored to its normal value What we are studying is the effect of desoxycorticosterone on the capacity of the kidney to excrete ammonia under conditions of mild dilution acidosis induced by a 2 ml water load or by a more severe type of acidosis induced by 100 milligrams of ammonium chloride The capacity of the kidney to put out ammonia and titratable acid under such conditions of acid stress has been more or less completely restored by the administration of desoxycorticosterone

Fremont Smith But in spite of that fact the glomerular filtration rate is restored and the blood flow to the kidney is restored?

Pitts I would infer that they were Actually blood flow and filtration rate were not measured in these rat experiments In dogs treated with DCA we have never seen any indication of restoration of diuretic response However we have not studied diuresis in cortisone treated dogs

Ralli Do you infer that there is an increased rate of reabsorption of water or how do you account for the absence of diuretic response?

Pitts I anticipate that cortisone would restore water diuresis in the dog This view is based on observations of Dr Gaunt on rats and of Dr Thorn on patients with Addison s disease In other words cortisone would reduce the excessive reabsorption of water which is characteristic of the adrenalectomized animal and Addisonian patient and which is responsible for the oliguria and failure of water diuresis

Ralli Dr Dumm and I* studied the effects of treating adrenalec

* Unpublished data

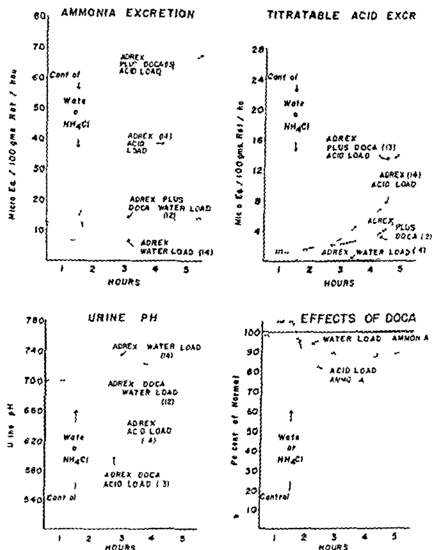


FIGURE 9 Comparison of the renal responses of untreated adrenalectomized rats and adrenalectomized rats treated with desoxycorticosterone to a mild and severe acid load

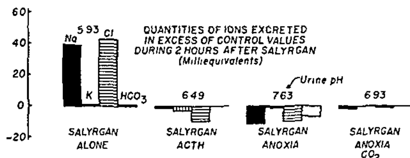


FIGURE 10 Comparison of the effects of salyrgan on the excretion of sodium chloride potassium and bicarbonate ions when administered alone following treatment with ACTH and during activation of the adrenals by anoxia

sodium and chloride loss by depressing some other ion absorptive mechanism However as illustrated in Figure 9 desoxycorticosterone in dosage of 0 8 to 80 micrograms per kilo reduces this pitressin induced sodium loss A dose of 0 8 micrograms per kilo reduces sodium loss by 20 microequivalents per minute whereas 80 micrograms per kilo reduces it some 66 microequivalents per minute It is evident that some 80 per cent of the sodium loss induced by pitressin can be antagonized by desoxycorticosterone

Hechter How do you inject DCA intravenously?

Pitts We dissolve it in sesame oil and homogenize it with saline in a small hand operated homogenizer We have observed no adverse effects from giving it intravenously We give cortisone in the same way

The implication of these experiments is that desoxycorticosterone stimulates a mechanism for absorbing sodium and chloride which is pitressin sensitive and which is therefore different from the ion exchange mechanism We infer that the adrenalectomized animal suffers no less than two distinctive deficits in ion absorptive capacity

Figure 10 illustrates this point in a different way* An intravenous dose of the mercurial diuretic salyrgan causes the loss of an excess of 40 mEq of sodium over and above the control rate during the two hours immediately following its administration Mercury by the way has no effect on the ion exchange mechanism i e it does not affect the rate of excretion of titratable acid and ammonia We then stumbled on the fact that if we caused an animal to inhale 6 per cent oxygen in nitrogen and gave that animal salyrgan no excess sodium loss occurred This abolition of salyrgan diuresis by anoxia might have been due to

* ROD D R d PITTS R F U publ l d d

tomized rats on high and low pantothenate diets with minimal doses of DCA or cortisone The hormones were given by injection the night before, the next morning the rats were hydrated to 5 per cent of their body weight The cortisone experiments were done eight days after adrenalectomy, and the DCA experiments were done in the same rats twenty two days after adrenalectomy Both groups of cortisone treated rats showed a slight increase in the per cent of water excreted as compared to the controls The DCA treated rats on the high pantothenate diet showed a marked increase in the per cent of water excreted although this was still below that of intact rats

Pitts Figures 9 and 10 illustrate a point which I should like to emphasize namely that reduction in activity of the ion transfer system by no means accounts for all of the difficulties of ion absorption which the adrenalectomized animal experiences

As shown in Figure 9 a small intravenous dose of pitressin of about 0 8 milliunit per kilo is chloruretic and natriuretic in the normal dog i e it causes the loss of sodium and chloride in the urine (19) Pitressin, however does not affect the acidity of the urine nor does it alter the rate of excretion of ammonia We infer therefore that pitressin does not alter the activity of the ion exchange mechanism but causes

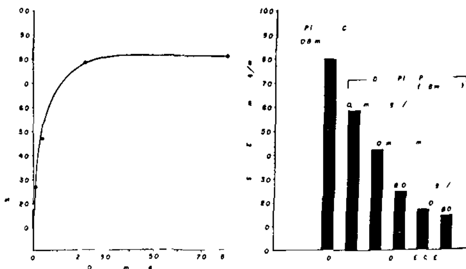


FIGURE 9 Antagonistic action of desoxycorticosterone on the increased sodium excretion produced by the intravenous administration of 0 8 m u of pitressin to the normal dog Reprinted by permission from Sartorius O W and Roberts K Effects of pitressin and desoxy corticosterone in low dosage on excretion of sodium potassium and water by normal dog *Endocrinology* 45, 275 (1949)

kidney of the adrenalectomized rat is very considerably reduced in comparison with that of the normal animal Jiminez Diaz (17) observed essentially the same type of thing But to my knowledge no one has studied the activity of glutaminase in the kidney of the adrenalectomized animal

The significance of that is that under normal circumstances probably two thirds or more of the urinary ammonia is derived from plasma glutamine the other third of the ammonia of the urine presumably comes from the circulating amino acids

I should like to think of the operation of the ion exchange mechanisms and the effects of adrenal hormones in terms illustrated in Figure 11 This Figure shows a cell from that region of the distal tubule in which these ionic exchanges are presumed to occur with one surface exposed to the tubular urine the other to interstitial fluid and blood A supply of hydrogen ions are continually available from carbonic acid produced in the cell by the hydration of carbon dioxide catalyzed by carbonic anhydrase Potassium ions are likewise concentrated in the cell by some unknown means probably common to all cells According to Berliner (21) cellular potassium ions or as we have shown (14 15)

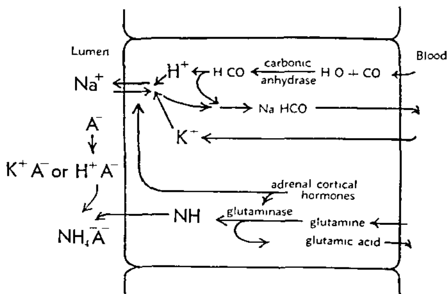


FIGURE 11 Diagrammatic representation of the probable sites of action of adrenal cortical hormones on the renal tubular mechanisms involved in the exchange of hydrogen ion potassium and ammonia for sodium

the associated respiratory alkalosis To determine whether or not alkalosis was a factor we caused the animal to breathe a gas mixture composed of 6 per cent oxygen 6 per cent carbon dioxide and 88 per cent nitrogen Despite even greater hyperventilation no respiratory alkalosis developed for the carbon dioxide maintained the alveolar tension of this gas at an elevated level Again salyrgan caused no diuresis In this experiment we noted a sharp decline in blood eosinophil level Because anoxia is known to activate the adrenal glands we gave salyrgan and ACTH giving the ACTH some three hours before the salyrgan Again the mercury diuresis was abolished

We interpret this experiment to mean that mercury depresses some ion absorptive mechanism which is different from the exchange mechanism Since adrenal hormones can antagonize the action of mercury they must act on some ion absorptive mechanism other than the exchange mechanism

Gellhorn Does CO alone have a similar effect?

Pitts No The administration of 8 per cent carbon dioxide in oxygen although leading to a severe respiratory acidosis has no effect on the action of salyrgan It neither increases nor decreases the diuresis

Perera In the adrenalectomized animal what changes appear in the glomerular filtration rate and renal plasma flow as the sodium intake is increased to very high levels?

Pitts I must hark back to some experiments of Dr Roemmelt (4) in which hypertonic saline was administered intravenously He observed that both the adrenalectomized and the normal dog respond with increased glomerular filtration rate and renal plasma flow Commonly however the response of the adrenalectomized animal is not as great as that of the normal animal but definitely there is an increase

Tremont Smith He starts at a lower level is that it?

Pitts No not necessarily We sustained our adrenalectomized animals on saline for some three to five days following cessation of hormone therapy At the time of the experiments they exhibited normal rates of glomerular filtration and renal blood flow Of course there is the possibility that in this period of three to five days all desoxycorticosterone had not been metabolized or eliminated I don t know how long it takes

Long Dr Pitts is there anything known about the glutaminase activity of the kidneys of the adrenalectomized animals? The source of most of the ammonia as I understand is due to the splitting of glutamine by glutaminase

Pitts I have no information on glutaminase

Of course Russell and Wilhelmi (20) in your laboratory showed about ten years ago I believe that the amino acid oxidase activity of the

mones on the inorganic and organic metabolism are not separate and distinct processes but are intimately associated one with another and that in the absence of these hormones the kidney is expressing the deficit in the only way a kidney can by a reduction in the rate of the processes that involve the greatest expenditure of energy and that is the separation of ions

White Since it is likely that this is a process dependent upon cell energetics one might expect that the response with cortisone might be slightly different from the type of response with DCA with respect to the correction of the deranged picture Dr Pitts has described in the adrenalectomized animal Is there a difference?

Long I was going to raise that question also because Dr Pitts dealt briefly with the different effects of the hormones I understood his conclusion to be that you could not distinguish between the effects of DCA and cortisone

Pitts And the whole adrenal cortical extract at least qualitatively

Hechter May I ask whether in the work of Russell and Wilhelmi (20) referred to L or D amino acids were used as substrates?

Pitts They used DL alanine and L(+) glutamic acid

Hechter Was the D or the L amino acid oxidase system affected?

Long I think they both were affected

White My recollection is that it was only the D because the D is so high in kidney and that is the one that is easy to measure The L is pretty well restricted to the liver

Hechter It seems most unfortunate that most hormonal effects on amino acid systems always seem to influence D amino acids

Bloch Transamination rather than oxidative deamination may be the quantitatively important pathway in the metabolism of L amino acids

White Except in the case of glutamic acid

Bloch There you have a specific enzyme which occurs in liver

Hechter When you say energetics Dr Pitts precisely what are you talking about?

Pitts I have to confess very frankly that I don t know what I am talking about However in the transfer of hydrogen ions from a low concentration in the cell to a high concentration in the urine and in the transfer in reverse of sodium in such a way as to lower its urine concentration to very low values a supply of energy is necessary Some source of energy which can be cycled into the system in some way is required Since one can affect both hydrogen and potassium transport by removing the adrenal glands it is likely that one is affecting their common link That link might be the place where energy is cycled into the system or it might be the energy cycling system itself

cellular hydrogen ions may be exchanged for sodium ions in the tubular urine Some stage reasonably the final one is common to exchange of both hydrogen and potassium ions for as Berliner has shown competitive inhibition of transport of these ions may be demonstrated Certainly the exchange of hydrogen ions for sodium involving as it does the development of a high hydrogen ion gradient across the lumenal membrane must involve the expenditure of energy The concentration of potassium in the urine at least as compared with the blood must likewise involve energy Presumably the common final and energy consuming step is the one affected by adrenal hormones In the absence of adrenal stimulation neither potassium nor hydrogen ions are properly concentrated in the urine

There is some reason for believing that ammonia diffuses into the urine as free ammonia and that its rate of diffusion is primarily controlled by the hydrogen ion concentration of the urine If this is true any reduction in the hydrogen ion concentrating capacity of the kidney would be reflected in a reduction in ammonia output Thus adrenal hormones would affect ammonia excretion secondarily and in consequence of their effect on the mechanism which exchanges hydrogen ions for sodium ions

However it is probable that adrenal hormones affect ammonia output in an even more intimate fashion Russell and Wilhelmi (20) have shown that kidney slices from adrenalectomized rats oxidatively deaminize amino acids less rapidly than do those from normal animals I would predict that glutaminase activity of the kidney might well be found to be similarly depressed If so then adrenal hormones would control the rate of formation of ammonia from glutamine as well as from amino acids

Long I think that is a very important deduction Dr Pitts because all of us have been searching for a common link between the effects of adrenal cortical hormones on electrolyte and on water metabolism From what we know of their effects we deduce that in some way or another they influence the metabolism of amino acids or of proteins When surveying all the deficiencies of the adrenalectomized animal it is continually striking that there appears to be a common denominator in the inability of various organs to supply energy at an adequate rate for their function This is certainly so in the working muscle and may also be the reason for the inability to form liver glycogen from non carbohydrate sources Now here it is brought together in the kidney where the relationship between the supply of energy the sources of that energy from organic materials and the effects upon the urine can be examined very clearly by these methods As a result of this I think we are obliged to deduce that the effects of the adrenal cortical hor

Hechter Wouldn t you say it is rather surprising Dr Long that the quantitative difference produced by adrenal cortical hormones is as small as it is approximately 2 per cent in terms of unit time judging from Dr Pitt s work?

Long Well it adds up though It adds up to an equivalent loss of 800 ml of fluid a day

Ingle Dr Pitts isn t it also true that the adrenally insufficient kidney is unable to rid itself of a high sodium load in a normal manner?

Pitts Yes but I don t know why

The data I shall now present bear on the question of whether or not there exists an antagonism between desoxycorticosterone and cortisone in relation to electrolyte excretion (8) Figure 12 gives data obtained in eight experiments on a single adrenalectomized dog For each experiment there is plotted in block form the per cent decrease in sodium excretion over a two hour period following the intravenous administration of the indicated dose of hormone In each instance hormone therapy was withdrawn and three to five days permitted to elapse during which time minimal signs of insufficiency developed

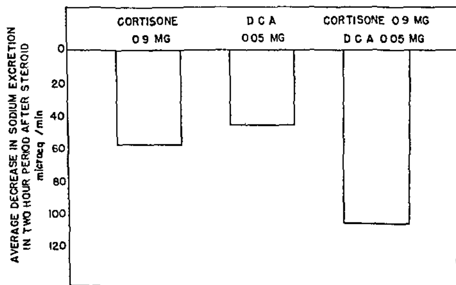


FIGURE 13 Comparison of the effectiveness of small doses of cortisone and of desoxycorticosterone administered separately and together in reducing sodium loss in an adrenalectomized dog Reduction in sodium loss is expressed in absolute terms of microequivalents per minute Reprinted by permission from Roberts K and Pitts R F The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog *Endocrinology* 50 51 (1952)

Tremont Smith Aren't enzymatic processes implicit in your unknown?

Pitts Yes

Pincus Have carbonic anhydrase inhibitors been given to adrenalectomized animals?

Pitts Not that I know of

Pincus That presumably would dissect out at least one phase

Pitts Only partially because this system can operate without adrenal cortical hormones and furthermore it can operate although at a reduced level of activity, in the presence of high concentrations of carbonic anhydrase inhibitors Hormones and enzymes merely whip up the activity of the transport system

Long I think that is a basic principle of endocrinology with which we all would agree that these are intrinsic properties of the cell and that the hormones are merely influencing their rate

Pitts Therefore one would not expect a qualitative difference One might expect a quantitative difference

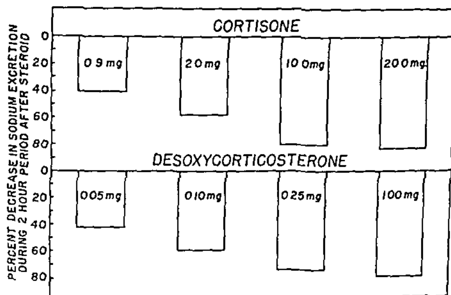


FIGURE 12 Comparison of the effectiveness of cortisone and desoxycorticosterone administered intravenously in reducing sodium loss in an adrenalectomized animal Reduction in sodium loss during the two hours immediately following the hormones is plotted in terms of per cent of that loss which would have occurred had no hormone been given Reprinted by permission from Roberts K and Pitts R F The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog *Endocrinology* 50, 51 (1952)

have observed none which demonstrated antagonism We must however remember that these are acute experiments and that they demonstrate only the immediate effects of these hormones when given intravenously Under these circumstances we cannot see any qualitative difference in the actions of cortisone and desoxycorticosterone only quantitative differences

Pincus This is the dog only? Have you done this with the rat?

Pitts No

Fremont Smith And this is sodium only?

Pitts This is sodium only the dog only Actually potassium measurements were made also but the immediate effects of either hormone on potassium are so inconstant from experiment to experiment that we could draw no conclusions concerning effects on excretion of this ion species

Loewi In acute experiments?

Pitts Yes acute experiments

Loewi How much time had elapsed after the administration of the hormones?

Pitts What was actually done was that urines were collected every twenty minutes If the rate of excretion of sodium during two or more control periods was roughly 100 microequivalents per minute the experiment was continued with the administration of hormone In the first period following hormone one would usually observe but slight reduction in sodium loss In subsequent periods sodium loss was sharply curtailed With the smallest dosages maximal effects were observed in an hour and evidence of wearing off was noted in about three or four hours However if one adds up sodium loss during the first two hours of each experiment and one always gets maximum effects within two hours and expresses that loss as a percentage of what would have occurred had no hormone been given we arrive at the figures presented in these charts

Fremont Smith I should like to go back to your very first statement about primary and secondary effects Would you specify in more detail what you mean by the difference between them

Pitts All right let s consider it in relation to the so called antagonism of action of desoxycorticosterone by cortisone The Addisonian patient maintained on minimal doses of desoxycorticosterone has in Thorn s (22) hands at least exhibited increased excretion of sodium i e negative sodium balance when given cortisone Now in Dr Sprague s hands (23)—I suppose we should let him speak for himself—I don t believe that has been observed

Sprague That s correct

Pitts Now assuming that the observations of Thorn Sprague and

The rate of sodium excretion was approximately 100 microequivalents per minute during the control periods of each experiment The intravenous administration of 0 9 mg of cortisone or of 05 mg of desoxycorticosterone produced about a 40 per cent reduction in sodium loss As the dose of each hormone was increased sodium loss progressively diminished A limiting value of around 80 per cent reduction in sodium loss was attained following the largest doses of the hormones This limit is the result of the delay of a half hour or so required for development of essentially complete absorption of sodium Throughout the entire range of dosage the ratio of activity of the hormones is about 20 1 DCA being about twenty times as effective as cortisone

If as shown in Figure 15 we choose doses of cortisone and DCA which are submaximal in their effects when individually administered and then give them together we get additive effects If on the other hand as shown in Figure 14 we give doses that individually produce essentially maximal effects and then combine them we get no increase also no antagonism We have tried all combinations of dosage and

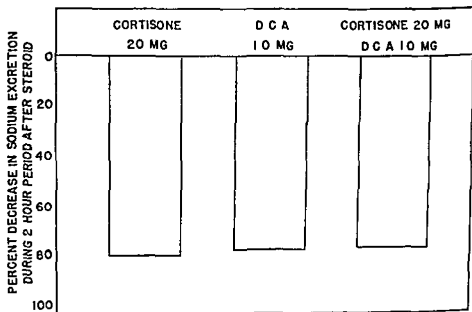


FIGURE 14 Comparison of the effectiveness of large doses of cortisone and of desoxycorticosterone administered separately and together in reducing sodium loss in an adrenalectomized dog Reprinted by permission from Roberts K and Pitts R F The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog *Endocrinology* 50, 51 (1952)

corticosterone by cortisone This study (24) was performed on a child with the nephrotic syndrome who was markedly edematous Throughout the whole experiment renal function was quantified day by day on the basis of the twenty four hour creatinine clearance with the idea that this clearance would give an indication of the time of onset of any marked change in renal function During the interval shown at the bottom of the chart ACTH was administered With the cessation of ACTH treatment the creatinine clearance increased sharply and just at that time the onset of diuresis occurred

I would argue that when one observes an antagonism of the sodium retention induced by desoxycorticosterone upon the administration of cortisone an increase in filtration rate has occurred This antagonism is fundamentally akin to the diuresis produced in the edematous child upon the administration of ACTH or cortisone It is true in this instance that the diuresis occurred when ACTH was withdrawn However diuresis has been observed during the administration of ACTH

Fremont Smith Dr Pitts I am not quite satisfied yet on the question of primary and secondary Do you mean by primary that the hormone when it has a primary action on renal function is acting directly on renal cells without any intermediary agent?

Pitts If it exerts its action on the kidney prior to any alteration which it may eventually produce in composition or volume of the body fluids then I say it is a primary action

Fremont Smith If it had a direct effect on glomerular filtration by acting on glomerular arterioles that would be a primary effect?

Pitts Yes

Fremont Smith But if it did this by indirection through another organ system that would be a secondary action?

Pitts That s right If the hypothesis is correct about cortisone increasing filtration rate and being responsible for the sodium diuresis then that effect of cortisone is not a primary effect on filtration rate It is secondary probably to some change in composition or volume of body fluids

Fremont Smith Which is not due to its direct effect on kidney cells?

Pitts I would say that if you want to study the primary effects of hormones on the kidney you almost have to do it in an acute experiment because just as soon as you administer the hormones over a period of time you produce changes and the effects may be secondary not primary

Hechter When Dr Gaunt spoke at the Laurentian Hormone Conference in 1950 among other things he mentioned the possible interrelationship between the antidiuretic posterior pituitary principles and some of the electrolyte effects observed in adrenalectomized animals

ourselves are all correct how can we reconcile these diverse findings? Is there a primary antagonism of desoxycorticosterone induced sodium absorption by cortisone, both hormones exerting their divergent effects on the same renal tubular cells? Arguing from our own acute experiments I would say no *However antagonism, or what has been called* antagonism by Thorn has been demonstrated only in relatively long term or balance types of studies I would hazard the guess that increased excretion of sodium in those instances where it has occurred following the administration of cortisone during desoxycorticosterone therapy is due to an increase in filtration rate In Sprague s patients this increase may not have occurred In Thorn s patients it may well have occurred If this guess is correct I would label this type of antagonism as secondary It would be secondary to the increased rate of glomerular filtration

I am indebted to Dr Henry Barnett for Figure 15 which constitutes such evidence as I have for believing that increased filtration rate may be the significant factor underlying the so-called antagonism of desoxy

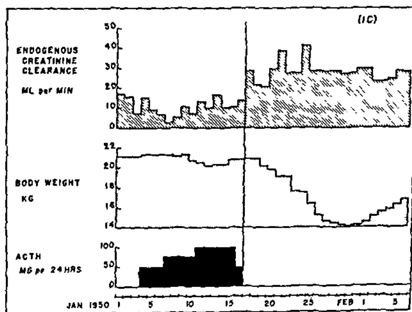


FIGURE 15 Effects of ACTH on renal function (24 hour endogenous creatinine clearance) and body weight in an edematous child with the nephrotic syndrome Reprinted by permission from Barnett H L *et al* Effect of adrenocorticotropic hormone on children with nephrotic syndrome II Physiologic observations on discrete kidney functions and plasma volume *J Clin Investigation* 30 227 (1951)

tunate preparation of growth hormone which he had could be completely corrected (25)

Long That may have contained thyrotropic hormone

Pitts I believe that he wangled himself out of the possibility of these other hormones in some way or other I have forgotten his method of doing it

Pincus I am a little puzzled by primary when I recall the results of ACTH administration to normal human subjects in which there is initially a greater sodium excretion rather than sodium retention Of course this varies with dosage but certainly with moderate doses there is initially a sodium excretion and the retention occurs later on What happens to the primary event under such circumstances? Is it overcome by the increase in filtration rate or something like that?

Pitts I would say we don t have the evidence at the moment to determine whether filtration rate has anything to do with this at all Perhaps estimations of filtration rate using even such a gross estimate as the twenty four hour creatinine clearance might provide us either with an answer in favor of or one which would convincingly remove that argument from the entire field

This question of primary and secondary effects of adrenal hormones is not at all complicated I am just not making myself clear as to what I mean by the distinction The animal in obvious adrenal insufficiency exhibits retention of urea retention of sulfate and retention of phosphate Is this due in any way to a primary depression of the mechanisms of excretion of urea or sulfate or phosphate i e increased tubular permeability permitting back diffusion etc? My answer to that would be no The insufficient animal loses sodium chloride and water The extracellular fluid volume is reduced and the circulating plasma volume shrinks *Glomerular perfusion pressure drops and filtration rate is* secondarily reduced Secondary to the decrease in plasma volume and even secondary to the resultant drop in filtration rate there occurs nitrogen sulfate and phosphorus retention Maintain plasma volume and filtration rate and the absence of hormone has no effect on the excretion of these substances

Selye What is known about the immediate electrolyte effects of adrenal cortical hormones following complete nephrectomy in such acute experiments?

Pitts I should point out that all deviations in composition of the body fluids following the administration of cortical hormones should not be considered to be the effects of these hormones on the renal excretion of ions The adrenal hormones assuredly alter the distribution of ions between the intracellular and extracellular fluid compartments In experiments of Dr Roberts summarized in Table V what must have

I was wondering to what extent this type of complicating factor might influence the primacy of these effects

Pitts I don t think I can answer your question but let me walk around it Every bit of evidence that I know is consistent with the view that there is a primary effect of adrenal hormones on the tubular cells to increase their absorption of sodium and that this effect occurs promptly I have tried to break down this primary action on sodium absorption suggesting that two different mechanisms were involved I indicated that the adrenal hormones act primarily to stimulate the exchange of hydrogen or potassium or ammonia for sodium I also pointed out that there was evidence of an effect on another sodium absorption mechanism

Hechter What about this other mechanism that you spoke of not involving ion exchange? Is that also primary ?

Pitts Yes I would say it is also primary It is a mechanism which is depressed by the administration of a mercurial diuretic Hence it is different from the exchange mechanism because mercury does not alter the reaction of the urine nor does it affect the excretion of ammonia The fact that ACTH or desoxycorticosterone antagonizes the sodium loss induced by mercurial diuretics suggests that these hormones affect a sodium absorptive mechanism different from the exchange mechanism

If you want to pin me down my guess is that these hormones act not only on the exchange mechanism presumably localized somewhere near the middle of the distal tubule but also on a sodium or chloride absorbing mechanism located somewhat more proximally in the renal tubule Whether actually in the proximal segment or in the more proximal part of the distal segment I cannot say That can only be decided by information as to where mercury acts

Ralli It might be that you are introducing an artificial differentiation in attempting to distinguish the primary and secondary effects As Dr Long has previously mentioned the intrinsic property of the cells is an important factor and the effect of a hormone is to stimulate a reaction within the cell Probably the direction of the reaction depends on the intracellular situation and this might be the basis as to whether the effect is primary or secondary

Hechter The question that I had in mind might best be answered by the results which follow corticosteroid administration in patients with diabetes insipidus or in animals wherein the pituitary has been removed Have such studies been done?

Pitts White has done studies in hypophysectomized dogs and has found that there is a marked reduction in glomerular filtration rate and in plasma flow which is only partially corrected as I recall by the administration of adrenal cortical hormones but which with one for

scarcely enough the behavior of the kidney was discouragingly capricious He found it difficult to keep a kidney viable and functional in all respects over a reasonable period of time And at the moment I would not hope to use the pump lung kidney preparation in an analysis of such small deviations in function as are produced by the presence or absence of adrenal cortical hormones

Our experience has not been the same as that of Dr Alfred Gillman who is likewise working on the pump lung kidney preparation He has experienced his greatest difficulty in maintaining reasonable rates of glomerular filtration yet has been able to maintain tubular secretory capacity in a satisfactory state Our difficulty has been exactly the reverse We have managed to maintain fairly good rates of glomerular filtration but tubular secretory and reabsorptive capacities rapidly deteriorate

Hechter How long is there evidence of glucose reabsorption in these isolated kidneys?

Pitts They absorb glucose throughout the experiment but measurements of absorptive capacity indicate reduced activity with passage of time

Hechter How long would you say the preparation is normal?

Pitts I cannot say that it was ever normal in our pump lung preparations We made control measurements with the kidney *in situ* and then switched it rapidly to the pump lung circuit and continued our measurements Values were always reduced As a matter of fact any function we chose to measure was reduced the minute we transferred the kidney to the pump lung circuit

Hechter Is there protein in the urine from the perfused kidney?

Pitts No the urine from the artificial kidney remained protein free in nearly all our experiments

Hechter And does it concentrate urea?

Pitts Urinary constituents are concentrated however the best concentration is observed early in the experiment

Selye Well it is certainly much easier to remove the kidney and study the rest of the body than to remove the kidney and study the kidney *in vitro* That is why I asked what you found in acute experiments after complete nephrectomy because whatever electrolyte change you find in the plasma could not possibly be mediated by the kidney

Pitts That I think illustrates our difference of approach We are primarily interested in observing how the adrenals affect renal function less so in how they affect the economy of the body as a whole You I imagine are more fundamentally interested in the latter question

Fremont Smith Dr Selye s question also bears on your approach because if you find changes taking place in the absence of the kidney

TABLE V

The Effects of Cortisone and Desoxycorticosterone on Plasma Concentration and Rate of Excretion of Potassium

Time mins	POTASSIUM Plasma mM /L	Urine mM /L	Urine μEq /min	Urine Flow ml /min
		Experiment 1		
Control	6 55	54	90 0	1 66
0		Cortisone 0 9 mg DCA 0 05 mg iv		
120	1 43	108	82 0	0 76
		Experiment 2		
Control	6 36	137	123 0	0 90
0		Cortisone 2 5 mg iv		
120	4 50	200	120	0 60

Reprinted by permission from Roberts K and Pitts R F The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog *Endocrinology* 50 51 (1952)

been extrarenal effects of cortical hormones are clearly evident Both experiments were performed on adrenalectomized dogs allowed to develop signs of moderate insufficiency by withdrawing therapy some five days previously In both instances despite rather high rates of excretion of potassium during the control periods plasma concentrations of potassium were distinctly elevated Within two hours after the administration of cortisone or of a mixture of cortisone and desoxycorticosterone plasma potassium dropped to a value within the range of normal In neither instance was this drop in plasma level dependent on an increased rate of excretion of potassium So the kidney is not all

Long Might it be due to a shift of water to the extracellular compartment?

Pitts We cannot say but if so it would require an expansion of the extracellular fluid compartment by half

Thorn Did these animals receive glucose?

Pitts These animals were getting nothing except the hormone

Long Is there any point these days Dr Pitts in attempting to use isolated kidney preparations particularly heart lung kidney preparations?

Pitts Last year Dr Kupfer worked in our laboratory on the pump lung kidney preparation He felt that a pump lung kidney would be better than a heart lung kidney because you could at least eliminate one variable the heart However the elimination of one variable was

about the action of adrenal hormones on the kidneys I merely wished to indicate that I knew it too However we were and are primarily interested in renal function and the actions of adrenal hormones constitute only one facet of the problem

In an attempt to simplify our experimental approach we got into immediate difficulty My view was that adrenalized animals are hard to maintain thus why not perform a bilateral adrenalectomy and study the animal immediately afterwards as an acute preparation thereby avoiding the trouble of caring for adrenalized animals However it became evident right at the start that marked shifts occurred in plasma concentrations of potassium sodium chloride and bicarbonate with no obvious renal cause for them at all One might get a marked drop in both sodium and potassium excretion and yet find the plasma sodium decreasing and the plasma potassium increasing We stopped that approach because it told us nothing about the effects of the adrenal hormones on kidney function

Ingle I would like to raise a general question Dr Pitts We talk about the adrenal cortex as regulating electrolyte balance but a distinction should be made between a regulatory role of an endocrine organ and the effect of its hormones upon a function I am not certain that the adrenal cortex is an important regulator of electrolyte and water balance although either a deficiency or an excess of its hormones has important effects upon the distribution and excretion of electrolytes and water There are important mechanisms regulating renal functions which operate independently of any change in adrenal cortex activity This can be shown by taking out the adrenal glands substituting for them a uniform intake of adrenal cortex extract and then subjecting the animal to situations which cause changes in electrolyte and water balance Insofar as these situations have been explored the renal adjustments in electrolyte and water metabolism go on almost if not entirely normally The presence of the cortical hormones may be required for normal renal functions but these adjustments can take place independently of any change in the secretory activity of the adrenal cortices

Pitts I think you have expressed the point very well I would amplify it along another line and carry it just a little further by stating that the kidney has a remarkable capacity to thumb its nose at the investigator You can create a disturbance for example by the continued administration of large doses of desoxycorticosterone but eventually the kidney compensates and the system comes into balance You can displace the level of plasma sodium and alter the volumes of the extracellular and intracellular fluid compartments but eventually and usually moderately rapidly the kidney undoes the damage which you

that is good evidence that they are secondary not primary effects It seems to me that the two could be complementary

Pitts We find changes in the presence of the kidney which are not due to the kidney

Selye There is a means of proving your point If it is really not due to the kidney not even due to possible renal hormones it would probably occur in nephrectomized animals

Loewi What would you measure?

Selye Potassium sodium and water in the blood

Loewi Would not the time be too short for the development of demonstrable changes in the blood? Should not one look for them in the urine?

Selye A nephrectomized rat especially an adult nephrectomized rat if it is given a comparatively low sodium and low protein diet will live for many days after complete nephrectomy You could compare the plasma values in a nephrectomized but otherwise untreated animal with that of a nephrectomized rat receiving corticoids

Loewi It will live for days?

Selye Oh yes It will live for forty eight hours without any precautions being taken It will live for many days if you give it a low sodium and low protein diet

Long It gets a little complicated doesn t it when the blood urea goes up in forty eight hours to 200 or 300 mg per cent?

Selye Is it less physiologic to take out the kidney and perfuse it?

Long No I don t think it is

Ingle Drs Kendall Nilson and I (26) published a paper some fourteen years ago showing an extrarenal effect of adrenal cortex extract and there are a number of other studies which show an extrarenal effect of the adrenal cortical hormones upon salt and water metabolism I do not quite agree with Dr Fremont Smith that if extrarenal effects exist there is no primary effect upon the kidney The kidney may still be an important site of action of these steroids which seem to be general tissue hormones Dr Conn has shown that the adrenal cortical hormones affect sodium excretion by the sweat glands There are similar changes in the concentration of electrolytes in saliva Cantarow and Rakoff (27) have shown that the rate at which sodium and chloride enter the peritoneal cavity of the dog following the intraperitoneal injection of glucose is greatly increased by 11 desoxycorticosterone

Pitts Yes I agree completely with Dr Ingle As a matter of fact I did not wish to imply that these data demonstrated anything new I thought everybody knew and agreed with the fact that you do get these effects independent of the kidneys But having spoken so extensively

surrounding towns when the central fire gets out of hand

Constellations of events in sequence complicate our experiments but that is the nature of nature and it is important to remember that although we try to isolate a single factor and find a cause for an event or a change philosophically and actually that can only be relatively true that it is really an unsound premise and that what one is dealing with at all times is a Gestalt a multicausal situation multicausal both in space and in time or sequence

There is no such thing as a characteristic response of an organism to any stimulus or disease It all depends upon the state of the organism Its response to the same stimulus may be and in fact will be diametrically opposite if the appropriate circumstances appropriate past history and past experience of the organism are provided It is certainly extremely useful and necessary in experiments to act as if single causality under certainly very sharply defined limitations operated but I think we need to remember if my thesis is at all right that that is something of an artificiality

Pitts Philosophically I suppose we do not differ greatly but I would look at the present state of affairs in my own meager understanding of the effects of adrenal hormones on renal function as one in which we are in the analytic dissecting stage We have nowhere approached the synthetic stage of putting all these things together That is really one reason why I emphasized the significance and importance of determining primary actions of adrenal hormones Eventually when we line up all of the primary actions and understand some of the secondary ones as well then we can begin to put the things back together again but I don t think we have reached that point yet

Vollmer Going back to the experiment shown in Figure 14 Dr Pitts do you think that the cortisone and DCA became available at about the same rate?

Pitts I can say that the rates at which they develop activity in stimulating tubular absorption of sodium the general declines of the curves of sodium excretion are not too greatly dissimilar

Vollmer They have the same peaks I was wondering whether the difference between the kinds of emulsion the DCA initially dissolved in oil and the cortisone in suspension might make a difference in the rates at which the steroids would go into the blood That could affect the validity of your 20 1 ratio

Pitts When you consider minimal doses of these hormones effects on sodium excretion reach a maximum in one to two hours and then disappear within the course of about four hours That is true of either one if you give minimal doses

Vollmer That seems to be good evidence that they do go in equally fast

have done One needs only to recall the diabetes insipidus like syndrome described by Loeb (28) as an example of re equilibration to overdosage of desoxycorticosterone And I would tend to agree with you that although the hormones modulate certain aspects of renal function the kidney has inherent within itself a remarkable capacity to regulate composition and volume of body fluids independent of so called hormonal regulatory mechanisms

Pereia As a corollary to what Dr Ingle said many of the effects of DCA are minimized or modified in the absence of adequate salt in the diet I wonder what would happen in your acute salyrgan experiments with DCA if the animals were given very little sodium?

Pitts Well of course if you salt deplete a normal animal say by the intraperitoneal administration of glucose and withdrawal of that glucose you can abolish the response of that animal to a mercurial diuretic It is an established clinical fact that in the presence of low plasma sodium and chloride patients will not respond adequately to mercurial diuretics Build up the plasma concentrations of sodium and chloride and the response returns to normal

Long I think Dr Engel and Dr Ingle have done a real service in introducing the term permissive function of the hormone in place of regulatory function

I should like to point out that some years ago Swingle (29) showed that the adrenalectomized animal may be allowed to go into insufficiency the serum sodium allowed to fall and then when put on a salt free diet and given cortical hormone recovers completely and for many weeks or months is apparently perfectly normal yet he continues to have abnormally low levels of serum sodium I think you are quoting the experiments with DCA in which serum sodium will rise and the amount in the urine will fall You have all the extremes between high and low serum sodium yet good health may be present with either The organism ultimately adapts itself to the new circumstances

Fremont Smith I should like to say a word on the sort of total hormonal stasis that in a sense we are talking about L J Henderson showed the seven interrelated factors which maintain the composition of the blood If I remember correctly he then showed that in conditions of abnormality (in acidosis or in administration of bicarbonate of soda or in conditions of anemia) the organism was able to throw in a series of secondary and tertiary homeostatic mechanisms when the basic or primary one failed It seems to me that is probably a situation which is generally true in the regulation of the organism we are not dealing only with those factors which we see for as soon as there is an abnormality sufficient either in intensity or in duration secondary factors become involved It is analogous to fire engines coming in from

ment this week on one of our completely adrenalectomized hypertensive patients Large doses of cortisone are being given by mouth over a short period of time in an attempt to discriminate between the primary effect of cortisone on glomerular filtration and those changes that might be secondary to the sodium chloride and water retention which follow the longer-continued administration of hormone intramuscularly The rapidity with which a peak hormone level is obtained by the oral administration of cortisone will permit one to make a decisive experiment

Pitts I want to go distinctly on record as pointing out that this is merely a hypothesis to explain two very divergent results I have absolutely not one shred of personal evidence that there would be an increase in filtration rate under those conditions in which an antagonism of hormones is observed I merely suggest it as something to reconcile these results

Thorn The reason I doubt it is that the first observations of this type were made in dogs on a very carefully controlled balance study and the phenomena occurred over the twenty four hour period This of course would come into the time relationship Dr Pitts indicates but I doubt seriously that a dose of cortisone of the magnitude which we used at that time would appreciably affect glomerular filtration rate *per se* although I cannot be certain

Pitts Just as soon as a dog or human drinks after he has had a dose of hormone then I think you have to acknowledge the possibility that the result may have been secondary to a change in composition or volume of the body fluids and the secondary result of some other renal action

Thorn I agree

Pitts I should like to hear what Dr Sprague might have to comment on the question of antagonism between DCA and cortisone

Sprague We have not seen anything that we would regard as evidence of antagonism between DCA and cortisone in patients with Addison s disease In our studies of electrolyte balance of patients with Addison s disease the addition of cortisone to a previous program of treatment with DCA produced no consistent change in sodium or chloride balance

Thorn You mean when there was no added salt retention?

Sprague No but the doses of cortisone used would not be expected to cause much additional retention of salt There was a question I wanted to ask Dr Pitts I believe it was brought up once before but did not get answered He presented some data indicating that cortisone caused a drop in plasma potassium in an adrenalectomized dog without increasing the urinary excretion of potassium Do you have any information about what happened to the potassium? Is it a matter of dilution or redistribution?

Pitts What I think might be a factor is this Is cortisone removed from the blood stream by all other tissues at a very much greater rate than is desoxycorticosterone? For that reason does it take more to attain any given concentration in the kidney? That I could not say but it occurs to me that there is a simple experimental approach merely to pass a catheter down into the renal artery and give the hormone directly into the renal artery

Pincus It would be extremely interesting

Ingle I don t think that we could disagree with Dr Pitts that cortisone is a weak sodium retainer Under certain conditions it does cause temporary sodium excretion There are at least two papers in the literature—I cannot give the references—showing that under certain conditions DCA also causes temporary sodium excretion Are you familiar with these studies Dr Pitts?

Pitts No

Sprague One of them is the paper of Soffer and associates (30) in which data are presented suggesting that DCA may cause a loss of sodium in patients with Cushing s syndrome I might say that we did not observe this effect of DCA in a dose of 20 mg daily in one patient with Cushing s syndrome whom we studied carefully

Conn That brings up a point on which I should like to enlarge In Thorn s experiments with the Addisonian maintained with DCA and then given cortisone in which Thorn interpreted the results as indicating the possibility of antagonism between the two hormones at the kidney level Dr Pitts now suggests that the addition of the cortisone increased plasma flow and that this as a secondary effect overcame the primary effect of DCA on the kidney

Pitts That is essentially the thesis I suggested that as a possibility

Conn It follows then that if instead of adding cortisone Thorn had added an increment of desoxycorticosterone he would have obtained *the same results as those which he observed when he added cortisone*

Pitts I should think it might act equally well especially if it produced an equal increment in filtration rate

Conn *If your thesis is correct the Addisonian should then excrete* sodium when the increment of DCA is given?

Pitts Yes

Conn That may be true in the normal who as we know is relatively resistant to large doses of desoxycorticosterone but the clinician is quite aware of the intense retention of sodium exhibited by the Addisonian who is given similarly large doses of DCA This seems like an entirely different effect from that obtained by Thorn when he added cortisone

Thorn Dr John Merrill and his group are carrying out an experi

Barnett and include the inulin clearance and the para aminohippurate clearance values observed while the patients were edematous prior to therapy and during the diuresis following ACTH (24) Prior to treatment filtration rates in these children were 8 14 and 10 ml per minute During the diuresis associated with the administration of ACTH filtration rate increased from 8 to 25 from 14 to 41 and from 10 to 28 ml per minute

Fremont Smith That increase in filtration rate alone could not possibly account for the diuresis could it? The diuresis is a change in urine volume of a different category of magnitude

Pitts Oh no this is altogether in a different category from loss of water This is ml per minute times 1440 minutes in a day No child lost that much edema in one day In patient N there is a difference of 25 and 8 namely 17 ml per minute times 1440 minutes

Thorn It is interesting to note that the untreated patient with Addison s disease has great difficulty in eliminating a test water load Granted that there may be several aspects of this problem one which immediately suggests itself is that the inability to excrete water rapidly suggests an excess of antidiuretic substance in the untreated patient with Addison s disease This is confirmed by the high antidiuretic assays which have been observed in both serum and urine Interestingly enough desoxycorticosterone treatment does not improve the ability to handle the water load whereas cortisone is capable of restoring the patient to normal in this respect Recently we have re investigated this problem particularly from a time dose viewpoint and have observed that a patient who on the previous day experienced a slow excretion of water following the test load when given 100 mg of cortisone by mouth at six o clock in the morning of the second test day is able to excrete water at a normal rate In other words within a matter of two to three hours the antidiuretic mechanism has been inhibited Our concept is that we have inhibited the production or action of an anti diuretic factor These observations may have some relevance to the diuresis which is induced in patients with the nephrotic syndrome One would expect that if the oliguria of the nephrotic syndrome were due primarily to an excess of antidiuretic factor a large dose of cortisone or intravenously administered ACTH should induce diuresis within the first twenty four hours (if this factor responded in the way that it does in patients with Addison s disease) The observation that a diuresis occurs much later than this suggests that the diuresis induced by cortical hormone treatment or withdrawal in patients with nephrotic syndrome likely includes some other mechanism

Pitts I should like to make the point that I would agree thoroughly with Dr Thorn that the effect of cortisone on water diuresis in the

Pitts I cannot tell you I don t know All I know is that it did not come out in the urine

Ralli May I ask one question about that slide that you showed on the child with a nephrotic syndrome (Figure 15)? In last year s Conference some work was presented on nephrotic children in which diuresis occurred regardless of therapy and in some instances seemed to bear no relation to the therapy Do you feel that the diuresis in this case you demonstrated was due to the ACTH effect or was it a spontaneous effect?

Long Would one of the clinicians like to rise to the occasion?

Sprague On that slide it looked like something more than coincidence I thought

Tremont Smith Did you get it repeatedly in that child? Did the child get edematous again and again after being given diuresis as a result of ACTH?

Pitts To be perfectly frank with you I borrowed those data from Henry Barnett

Perara In our experience as well as that of others 30 or 40 per cent of nephrotic patients will have a diuresis on withdrawal of ACTH or while it is still being administered If increases in filtration rate happen consistently and are so important then one must explain the failure of diuretic response in the majority of patients

Pitts The data summarized in Table VI are from studies of Dr

TABLE VI

Renal Functions in Children with the Nephrotic Syndrome

Patient	Clearances Inulin ml /min		Clearances PAH ml /min		Ratio C_{IN}/C_{PAH}	
	A	B	A	B	A	B
N	8	25	68	94	0 11	0 27
B	14	41	143	197	0 10	0 21
C	10	28	71	119	0 14	0 24

A with edema before ACTH

B during diuresis following ACTH

Reproduced by permission from Barnett H L *et al* Effect of adrenocorticotrophic hormone on children with nephrotic syndrome II Physiologic observations on discrete kidney functions and plasma volume *J Clin Investigation* 30 2 7 (1951)

18 WHITE H L Some pituitary and adrenal influences on renal function *Renal Function* Bradley S E Editor Trans Second Conf New York Josiah Macy Jr Foundation 1950 (p 79)
19 SARTORIUS O W and ROBERTS K Effects of pitressin and desoxycorticosterone in low dosage on excretion of sodium potassium and water by normal dog *Endocrinology* 45 *273 (1949)*
20 RUSSELL J A and WILHELMI A E Metabolism of kidney tissue in adrenalectomized rat *J Biol Chem* 137 *713 (1941)*
21 BERLINER R W KENNEDY T J JR and ORLOFF J Relationship between acidification of urine and potassium metabolism *Am J Med* 11 *274 (1951)*
22 THORN G W ENGEL L L and LEWIS R A Effect of 17 hydroxycorticosterone and related adrenal cortical steroids on sodium and chloride excretion *Science* 94 *348 (1941)*
23 SPRAGUE R G *et al* Metabolic effects of synthetic compound E (17 hydroxy 11 dehydrocorticosterone) in two patients with Addison s disease and one with coexisting Addison s disease and diabetes mellitus *J Clin Investigation* 28, *812 (1949)*
24 BARNETT H L *et al* Effect of adrenocorticotropic hormone on children with nephrotic syndrome II Physiologic observations on discrete kidney functions and plasma volume *J Clin Investigation* 30 *227 (1951)*
25 WHITE H L HEINBECKER P and ROLF D Enhancing effects of growth hormone on renal function *Am J Physiol* 157 *47 (1949)*
26 INGLE D J NILSON H W and KENDALL E C Effect of cortin on concentrations of some constituents of blood of adrenalectomized rats *Am J Physiol* 118 *302 (1937)*
27 RAKOFF A E and CANTAROW A Influence of certain steroid hormones on diffusion of chloride into peritoneal space *Endocrinology* 30 *816 (1942)*
28 RAGAN C *et al* A syndrome of polydipsia and polyuria induced in normal animals by desoxycorticosterone acetate *Am J Physiol* 131 *73 (1940)*
29 SWINGLE W W and REMINGTON J W Role of adrenal cortex in physiological processes *Physiol Rev* 24 *89 (1944)*
30 SOFFER L J *et al* Utilization of intravenously injected salt in normals and in patients with Cushing s syndrome before and after administration of desoxycorticosterone acetate *J Clin Investigation* 23 *51 (1944)*

adrenalectomized animal or in the patient with Addison s disease is certainly due to some effect of cortisone either in reducing the quantity of circulating antidiuretic hormone or in reducing the effectiveness of the action of antidiuretic hormone on the kidney and is not at all concerned with filtration rate

REFERENCES

1 Gaunt R The adrenal cortex in salt and water metabolism *Recent Progr Hormone Research* 6, 247 (1951)

2 Talbott J H *et al* Renal function in patients with Addison s disease and in patients with adrenal insufficiency secondary to pituitary pan hypofunction *J Clin Investigation* 21, 107 (1942)

3 Waterhouse C and Keutmann E H Kidney function in adrenal insufficiency *J Clin Investigation* 27, 372 (1948)

4 Roemmelt J C Sartorius O W and Pitts R F Excretion and reabsorption of sodium and water in adrenalectomized dog *Am J Physiol* 159 124 (1949)

5 Lotspeich W D Effect of adrenalectomy on renal tubular re absorption of water in rat *Endocrinology* 44 314 (1949)

6 Gaunt R Birnie J H and Eversole W J Adrenal cortex and water metabolism *Physiol Rev* 29, 281 (1949)

7 Harrison H E and Darrow D C Renal function in experimental adrenal insufficiency *Am J Physiol* 125 631 (1939)

8 Roberts K and Pitts R F The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog *Endocrinology* 50 51 (1952)

9 Thompson D D and Pitts R F Effects of alterations of renal arterial pressure on sodium and water excretion *Am J Physiol* 168 490 (1952)

10 Loeb R F *et al* Electrolyte balance studies in adrenalectomized dogs with particular reference to excretion of sodium *J Exper Med* 57, 775 (1933)

11 Harrop G A Nicholson W M and Strauss M B Studies on suprarenal cortex influence of cortical hormone upon excretion of water and electrolytes in suprarenalectomized dog *J Exper Med* 64 233 (1936)

12 Walker A M *et al* Collection and analysis of fluid from single nephrons of mammalian kidney *Am J Physiol* 134 580 (1941)

13 Smith H W *The Kidney Structure and Function in Health and Disease* New York Oxford Univ Press 1951

14 Pitts R F Acid base regulation by the kidneys *Am J Med* 9 356 (1950)

15 ——— Renal excretion of acid *Federation Proc* 7 418 (1948)

16 Atchley D W *et al* On diabetic acidosis detailed study of electrolyte balances following withdrawal and reestablishment of insulin therapy *J Clin Investigation* 12 297 (1933)

17 Jiminez Diaz C Death in Addison s disease (functional renal failure) *Lancet* 2, 1135 (1936)

found that neither effect could be obtained if a similar stimulus was applied directly to the anterior pituitary gland itself

About this time 1947 Dr Harry Colfer from the Mayo Clinic and Dr Jack de Groot from Amsterdam came to Cambridge and in collaboration with these workers the following experiments were performed First rabbits were exposed to emotional stress stimuli (immobilization and subcutaneous faradism) and it was found that such a procedure resulted in a lymphopenia in these animals as had been shown previously in other species (2) This lymphopenic response was very similar to that observed by Dougherty and White (3) following injection of ACTH The view that an emotional stimulus in the rabbit excited ACTH secretion by the anterior pituitary received strong support when it was found that hypophysectomy abolished the lymphopenic response although lymphopenia still occurred in the hypophysectomized in response to injections of crude anterior pituitary extract or of purified ACTH Denervation of the adrenal glands did not modify this response to emotional stress The way was then open to see whether lesions of the hypothalamus would block this response to emotional stress and such was found to be the case Lesions extending laterally in the posterior part of the tuber cinereum or in the mammillary body were observed to diminish or abolish the emotional lymphopenia Lesions restricted to the mid line of the tuber cinereum or lesions just posterior to the optic chiasma or in the more dorsal regions of the hypothalamus did not affect the response The stress lymphopenia was also blocked by a lesion in the anterior pole of the pituitary but not elsewhere in the gland (posterior part of the pars distalis pars intermedia or infundibular stem) (4) It may be asked whether the hypothalamic lesions acted by blocking some sympathetic mechanism concerned with release of epinephrine and so prevented the lymphopenia to stress Two facts argue against such an idea *(a)* that the lesions in the posterior part of the tuber cinereum are removed from the areas of the hypothalamus found by Magoun Ranson and Hetherington to be active in inducing adrenin secretion (5) and *(b)* that a continuous path could be traced from the mammillary body via the tuber cinereum into the pituitary gland

The reverse experiment electrical stimulation of the same structures in the unanaesthetized quiescent rabbit was performed to see whether a lymphopenia attributable to ACTH secretion might be so produced It was found that stimulation of the mammillary body or the posterior part of the tuber cinereum did produce a lymphopenic response similar to that following emotional stress or injection of ACTH but that stimulation of the zona tuberalis (a region where lesions blocked the emotional response) or other regions of the pituitary gland did not result

THE HYPOTHALAMUS AND REGULATION OF ACTH SECRETION

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THE FACTORS WHICH control the release of ACTH from the anterior pituitary gland have received much attention in the past few years At the moment there is a considerable body of evidence underlining the importance of three such factors

a) The systemic blood level of the adrenal cortical hormones which has been studied in detail by Dr George Sayers and his colleagues

b) The systemic blood level of epinephrine which has been investigated by Dr Long and his collaborators at Yale and by Dr Martha Vogt in England

c) The control exerted by the hypothalamus which has been studied by Dr David Hume in Boston and by myself and co workers in Cambridge

I should like first to give briefly the results of our own work and then to consider the apparent discrepancies and points of argument when compared with the results of others In 1935 our approach to the problem of the control of anterior pituitary secretion was influenced by the embryological development of the pituitary gland The anterior lobe of the pituitary is derived from Rathke s pouch which migrates from the stomodaeum into the cranium to make contact with the floor of the third ventricle It has now been determined that in many species two small evaginations of Rathke s pouch the lateral lobes constantly establish contact with the hypothalamus or the neurohypophysis and form in the adult gland the pars tuberalis and probably also the zona tuberalis (1) If this mode of development has any functional significance it seems likely that it would be in relationship to the control of anterior pituitary activity An investigation was therefore undertaken to see whether electrical stimulation of the hypothalamus would result in increased hormonal secretion by the anterior pituitary It was found that remote control stimulation of the tuber cinereum in the conscious rabbit may result in gonadotropic secretion as demonstrated by the occurrence of ovulation or in the secretion of some substance which antagonized the effect of injecting small doses of insulin It was also

with this method have been highly inconsistent After this operation in rats for example Dempsey and Uotila (11) and Dempsey and Searles (12) found that some animals exhibited normal reproductive processes while Westman and Jacobsohn (13) and Greep and Barnett (14) reported gonadal atrophy The effects of stalk section on the adrenal glands of the rat would appear to be equally variable for Cheng Sayers Goodman and Swinyard (15) reported adrenal glands of normal weight and Barrett and Greep (16) atrophic adrenal glands (about 50 per cent normal weight) following this procedure It was thought possible that the varied results obtained by different workers following pituitary stalk section might be due to different degrees of regeneration of the portal vessels and it was found (also in rats) that *(a)* section of the stalk by the subtemporal route may be followed by marked regeneration of the hypophyseal portal vessels across the site of section and *(b)* that the resumption of gonadotropic secretion by the stalk sectioned pituitary could be correlated with portal vessel regeneration and that if this regeneration was prevented by insertion of plates between the cut ends of the stalk atrophy of the gonads and reproductive tract followed (17)

Pitts May I ask how you get such nice injections of the portal system?

Harris The injections at first gave us some difficulty We found it was a disadvantage to perfuse the system with saline or nitrite solution but rather better to inject the ink straight into the vascular system with the heart still beating That seemed to give the best results From the work of Cheng *et al* (15) and of Barrett *et al* (16) it seems likely that a correlation exists between regeneration of the portal vessels and the maintenance of adrenal gland weights (or ACTH secretion) following pituitary stalk section Barrett and his co workers sectioned the pituitary stalk in rats by the parapharyngeal route trephining the skull immediately below the stalk so that after operation the two ends of the stalk became involved in scar tissue and portal vessel regeneration was reported not to occur Cheng and his co workers also used a parapharyngeal approach but trephined the skull immediately below the pituitary gland split the anterior lobe at the isthmus and sucked out the neural lobe between the two halves of the pars distalis In this case the portal vessels were not sectioned and it seems likely that this fact may be correlated with the normal weights of the adrenal glands observed by these workers When investigating the effects of pituitary stalk section on anterior lobe function it is of great advantage to use a temporal approach This method enables the operator to encourage portal vessel regeneration at a later date by leaving a smooth meningeal bed for the sectioned stalk or to prevent such regeneration by inserting a plate between the cut ends of the stalk

in lymphopenia The stimulus used had a spread of not more than 0 5 mm so far as the unmyelinated fibres of the hypothalamus were concerned The *interpretation placed on these results was that lesions or* stimulation of the mammillary body or posterior part of the tuber cinereum affected some neural pathway in the hypothalamus active in transmitting an emotional stimulus to the region of the median eminence of the tuber cinereum but that from the median eminence to the pars distalis of the pituitary gland the stimulus was transmitted humorally via the hypophyseal portal vessels of the pituitary stalk This hypothesis would explain why lesions which involved the zona tuberalis (a region of the gland traversed by the portal vessels) blocked an emotional lymphopenia and electrical stimulation of this same structure did not elicit a response

Pincus Could you also have a possible localization of ACTH producing cells in this portion where the lesion was effective?

Harris The lesion here would abolish the lymphopenic response by obliterating all the tissue that formed ACTH I suppose that is a possibility

Pincus I doubt that there is much evidence for it

Harris The anatomical basis for the first suggestion is well founded The anatomy of the hypophyseal portal vessels has been described by *others Popa and Fielding (6) Wislocki and King (7) Green and* Harris (8) and more recently in a detailed study by Green (9) Here it need only be mentioned that in all forms from amphibians to man Green found that a true portal system of blood vessels exists along the pituitary stalk These vessels begin as a capillary plexus (*primary* plexus) in the median eminence of the tuber cinereum pass as relatively large trunks down the pituitary stalk and break up into the sinusoids of the pars distalis of the gland The direction of blood flow in these vessels is from the median eminence of the tuber cinereum to the pars distalis of the pituitary (10)

Hechter Are those the only blood vessels which supply the anterior pituitary?

Harris These portal vessels form a fair proportion of the total blood supply of the anterior lobe but then varying to some extent with the type of creature there is also a systemic arterial supply which comes directly off the internal carotid In the case of the pituitary gland of the rabbit for example there is a direct comparison with the circulation of the liver that is the systemic supply from the carotids the portal vessels and then the systemic venous drainage

Another technique that has been much used in investigating control of anterior pituitary secretion has been the observation of pituitary function following section of the pituitary stalk but the results obtained

ovaries reproductive tract adrenals and thyroid appeared normal (It is perhaps of passing interest that pituitary tissue obtained from new born rats would maintain apparently normal estrous cycles in the hypo physectomized mother rat within one or two weeks of operation that is long before the young rats would have reached puberty Similar experi ments on another group of animals showed that male pituitary tissue was effective in maintaining estrous cycles and pregnancy if transplanted into female recipients)

In both groups of animals the completeness of hypophysectomy was checked by careful microscopic examination of serial sections through the whole extent of the hypophyseal capsule In both groups the grafted pituitary tissue had acquired a good blood supply (as demon strated by perfusion of the vascular tree with India ink) and there was no apparent difference in the vascularity or size of the grafts in the two cases The main vessels of supply to all grafts were cerebral ves sels but the median eminence grafts were supplied by the hypophyseal portal vessels which was not the case for the temporal lobe grafts Cytologically the median eminence grafts were well differentiated acidophile basophile and chromaphobe cells were seen The temporal lobe grafts consisted of small cells uniform in size

Thorn May I ask how long the cells in the pituitary were stained? Did they remain stained indefinitely as far as you know in the transplant?

Harris We followed most of these animals for about six weeks after transplantation although some of them were followed for many months There appeared to be no difference

Hechter With respect to the control group do you have any evidence whether those pituitary implants took?

Harris In the control group the transplanted pituitary tissue seemed to be as large if not larger than in the experimental group and so far as could be seen the vascular bed of the temporal lobe grafts seemed to be as great as that of the median eminence grafts

Tremont Smith You say those cells were differentiated In other words they showed the characteristic chromophil and chromophobe types?

Harris Yes in terms of the transplants under the median eminence but not under the temporal lobe

It seems clear that in these experiments the apparently normal func tion of anterior pituitary tissue grafted under the median eminence is correlated with the anatomical site of the graft There are at least three possible explanations as to why such grafts under the median eminence stand in marked contrast to grafts in other sites in the body

a) The vascularization of tissue grafted under the median eminence

Following the above experiments it appeared safer to draw conclusions regarding hypothalamic control of ACTH secretion from a study of transplanted anterior pituitary tissue rather than from a study of the normally situated gland which had been disconnected from the hypothalamus by stalk section Cheng Sayers Goodman and Swinyard (18) made auto and homo transplants of anterior pituitary tissue into the eye spleen or sella turcica of adult hypophysectomized rats They found that With the exception of one animal with a sellar graft the rats with pituitary transplants were found to have atrophic adrenals The decrease in weight of the adrenals was in most instances as great as that which occurred in the hypophysectomized animals without pituitary grafts It was also found however that the transplanted pituitary tissue would discharge ACTH in response to injected histamine although the amount discharged seemed less than normal The testes in two rats of this series were examined and found to be atrophic McDermott Fry Brobeck and Long (19) made homologous grafts of pituitary tissue to the anterior chamber of the eye of hypophysectomized male rats The adrenal glands of these animals underwent partial atrophy and the testes became completely atrophic A painful stimulus (subcutaneous injection of hypertonic saline) or injection of epinephrine (subcutaneously or into the anterior chamber of the eye containing the transplant) resulted in an eosinopenia in these animals

In view of the suggestion that normal function of the anterior pituitary gland depends on the hypophyseal portal vessels (20 8) it was decided to investigate the function of anterior pituitary grafts placed in a position where they might become revascularized by these vessels This work was performed in collaboration with Dr Dora Jacobsohn of the University of Lund Sweden (21) Grafts were made in different sites into 151 hypophysectomized adult rats and the results obtained in two groups of these animals were as follows

a) *Control group* Ten hypophysectomized female rats had grafts of pituitary tissue obtained from their own young placed in the subarachnoid space under the temporal lobe of the brain Postoperatively estrus was not observed to occur in any of these animals and post mortem the ovaries reproductive tract adrenals and thyroid were found to be atrophic

b) *Experimental group* Twelve hypophysectomized female rats had grafts of pituitary tissue obtained from their own young placed in the subarachnoid space under the median eminence Postoperatively estrous cycles of normal rhythm returned to all animals On placing with male rats six of these animals became pregnant and delivered living litters after apparently normal pregnancies and parturition Milk secretion by the mammary glands also appeared normal Post mortem the

that sound and immobilization induced a marked fall of the eosinophils in intact but not in grafted animals Fortier suggests that these results indicate a dual regulation of ACTH release one purely humoral in response to systemic stress stimuli and the other probable neurohumoral mediated by the hypothalamo hypophyseal neurovascular pathway and coming into play under the influence of nervous or emotional stimuli

There is little reason to doubt that the factors governing the secretion of ACTH are similar to the factors governing the secretion of the gonadotropic (and perhaps the other tropic) hormones In both cases the hormonal output is influenced by such factors as (*a*) electrical stimulation of the tuber cinereum but not of the anterior pituitary gland directly (*b*) administration of the target organ hormone (adrenal cortical hormone and estrogen) (*c*) transplantation of the pituitary gland to a site remote from the sella turcica (*d*) changes in the external environment (light temperature etc) (*e*) emotional disturbances (*f*) administration of barbiturate drugs The available evidence would indicate that the secretion of ACTH and gonadotropic hormone is normally maintained and regulated by the hypothalamus acting via the hypophyseal portal vessels It is likely that the systemic blood level of adrenal cortical hormone (and estrogen) exerts a fine control by means of a feedback to the hypothalamus Since however stress may lead to increased amounts of cortical hormone in the urine the influence of such a mechanism can only be of subsidiary importance under many conditions of stress

This seems to me to be the simplest explanation of the facts (Figure 16) that the secretion of ACTH by the anterior pituitary gland is largely controlled by some hypothalamic mechanism acting by means of these hypophyseal portal vessels passing down the pituitary stalk ACTH stimulating the adrenal cortex and then adrenal cortical hormone (and perhaps adrenalin) acting as some feedback mechanism more probably on a hypothalamic region rather than directly on the pituitary gland itself I think the evidence derived from pituitary transplants is significant in this respect If anterior pituitary tissue is removed from the hypothalamus then this mechanism fails but if it is in relationship with the hypothalamus then adrenal glands within the normal weight range may be maintained That I think would be evidence arguing that the systemic blood level of the adrenal cortical hormone or adrenalin affects some hypothalamic rather than anterior pituitary mechanism

Gellhorn Does that mean that the secretion of ACTH induces an increased secretion of adrenalin from the adrenal medulla?

Harris No this diagram is supposed to mean that ACTH causes increased secretion of adrenal cortical hormones but two factors that possibly react upon the hypothalamus are put in the one diagram

may be greater than that of tissue grafted into other sites and the return of functional activity may be related to the total blood supply Although the vascular tree of the grafts under the temporal lobe appeared to be as great as in the case of the grafts under the median eminence it is still possible that the rate of blood flow may have been greater in the vessels of the median eminence grafts However it would seem probable that in this case the functional difference between the two groups of animals would have been one of degree only

b) Secretomotor nerve fibres from the hypothalamus may regenerate into the median eminence grafts and be responsible for the return of function It is however very doubtful whether nerve fibers do pass from the hypothalamus into the pars distalis of the normal pituitary gland (9) and even more doubtful that if such fibers exist they would regenerate after section Fisher Ingram and Ranson (22) and Magoun Fisher and Ranson (23) demonstrated that after interruption of the supraopticohypophyseal tract regeneration of nerve fibres does not occur This might be expected from the fact that these are nerve fibres of the brain and not of a peripheral nerve

c) The blood carried by the hypophyseal portal vessels has some stimulant effect on anterior pituitary tissue which is not a property of blood in other vessels This hypothesis seems to be the only one which will explain the facts

It appears then that ACTH secretion by grafted anterior pituitary tissue is only retained at a normal level if the tissue is vascularized by the hypophyseal portal vessels and that if the tissue is in a site remote from the hypothalamus partial or complete adrenal atrophy occurs This indicates that variations in the blood levels of epinephrine or of adrenal cortical hormone do not stimulate ACTH secretion by direct action on anterior pituitary cells

There is little evidence whether the factors which control ACTH secretion under optimum quiescent conditions are the same as those concerned with the increased production of ACTH under conditions of stress It would be of much interest in this respect to compare the response of pituitary grafts under the median eminence with response of pituitary grafts elsewhere to stress stimuli of different types Fortier* has performed a similar type of experiment He exposed groups of normal rats and hypophysectomized rats with anterior pituitary transplants in the eye to varied types of stress stimuli (*a*) systemic (epinephrine cold histamine) (*b*) emotional (immobilization) and (*c*) sensory (sound) It was found that epinephrine cold and histamine brought about a definite eosinopenia in both normal and grafted animals but

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* Personal communication

Gellhorn What is the relation? I can see that the increased secretion of ACTH increases the amount of adrenal cortical hormones circulating But how does that affect the adrenalin level in the blood?

Harris The diagram is not supposed to indicate that it does affect the adrenalin level in the blood These two factors the systemic blood level of adrenal cortical hormones and the systemic blood level of adrenalin were inserted to show a reaction back on some structure in the region of the hypothalamus or pituitary gland which in turn affected ACTH secretion

Gellhorn You probably agree Dr Harris that the level of adrenalin cannot be altered by any other mechanism than by the sympathetico adrenal discharge

Harris Yes I am in thorough agreement

I should like to list some of the problems that seem of importance First it is important to study the effect of lesions in the hypothalamus in much more detail There are two possibilities in this connection One is that such lesions can block some sympathetic medulla mechanism and the other is that they block some mechanism directly affecting the anterior pituitary gland The lesions in our animals seem to be too near the mid line to involve any sympathetic mechanism in the hypothalamus Secondly there is the problem of whether the results of the graft experiments can be explained on the question of the total blood supply of the grafts in the two regions under the hypothalamus or elsewhere whether there is a possibility of secretory nerve fibers growing back from the hypothalamus into the grafts which I think unlikely whether there is some effect secondary to thyroid activity on the grafts of the two different types or whether the results are a specific effect of the portal blood vessels

Then third there is the question of the evidence regarding the basal secretion of ACTH versus the increased secretion under conditions of stress

There is also another point If the anterior pituitary is truly dependent upon some chemical transmitter carried by the hypophyseal portal blood vessels it raises the question as to how many chemical or humoral transmitters in those blood vessels it is necessary to postulate and that in turn raises the question as to how many separate hormones are in fact secreted by the pituitary gland into the blood stream I am not sure of the evidence on this point and I shall be grateful for any information as to how many hormones are liberated from the pars distalis into the blood stream because it seems to me very germane to the question of humoral transmitters

Long Thank you Dr Harris

Gellhorn Dr Harris I should like you to clarify your position with

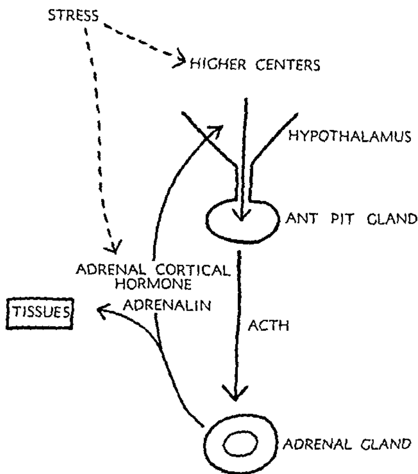


FIGURE 16 Diagrammatic representation of a possible mechanism of control of ACTH secretion *Under optimal quiescent conditions it* is believed that the basal output of ACTH is under hypothalamic control mediated by the hypophyseal portal vessels of the pituitary stalk According to the type of stress this basal output may be increased either by *(a) an effect on the blood level of adrenal cortical* hormones and adrenalin and/or *(b)* an effect mediated by the hypothalamus and pituitary stalk On the data available it is thought likely that the blood level of adrenal cortical hormones and adrenalin affect anterior pituitary activity via the hypothalamus and not by an action directly on anterior pituitary cells Reprinted by permission from Harris G W Neural control of the pituitary gland II The adenohypophysis with special reference to the secretion of ACTH *Brit M J 2, 627 (1951)*

sympathetico adrenal mechanism comes first This may be contrary to your experience with the rabbit but I wonder whether the choice of this animal was not unfortunate in this particular respect

I have no criticism whatever of the beautiful experiments which you have presented but the rabbit is apparently an animal in which the sympathetico adrenal discharge is unimportant I don t think it was an accident that Cannon for instance carried out most of his studies on the cat and that other investigators preferred in addition to cats dogs It is known for instance that a condition such as severe exercise leads to depletion of the adrenal medulla as far as adrenalin is concerned in the cat and in the dog but it does not do so in the rabbit So I am not at all surprised that relatively mild stimuli so called emotional stimuli restraint etc are not productive of these results in this particular animal

I should like to support the thesis that the initiating mechanism in the liberation of ACTH is the sympathetico adrenal discharge although we have to admit that in various species possibly in various individuals if we want to apply it to the human the reactivity of the centers of the sympathetico adrenal system will show wide variations

Harris I would not maintain that our experiments with adrenalin injections in the rabbit had very wide significance but Dr Hume I believe has dealt with sympathectomized dogs and found no difference in the eosinopenic response to various stimuli

With regard to adrenalin I think it is difficult to conceive of it as being more than of subsidiary importance as shown by the graft experiments If the pituitary is removed from the hypothalamic region then adrenal atrophy follows although adrenalin is presumably being secreted normally in these animals Although there is viable well vascularized pituitary tissue present in large amounts adrenal atrophy occurs If this was a mechanism involving only adrenalin and pituitary tissue and the interreaction between the two then the integrity of the adrenal cortex should be maintained

Gellhorn I fully agree with that I think that there are two mechanisms and one is more sensitive than the other And of course I spoke only of the observations under stress That has nothing whatever to do with the physiological mechanism which maintains the adrenal cortex in a state of function when stress is not present I would not see any reason why the sympathetic adrenal mechanism would be called into action under these conditions I certainly believe that the hypothalamus and the pituitary function without the intervention of adrenalin under resting conditions

Harris We are probably in agreement I would not deny for a moment that adrenalin may be one factor involved in the stress responses

regard to the role of the adrenalin secretion It is quite clear to me from your publications but for further discussion it would be helpful

Harris The only observations we have made on the significance of adrenalin secretion were these that adrenalin injected intravenously *into rabbits in small doses (1 ml of 1/150 000 solution of adrenalin* hydrochloride) did not in our hands produce a constant lymphopenia We had a series of rabbits in which the adrenal medulla was denervated and those rabbits reacted to emotional stimuli in the same way as they had done before operation It seemed to us therefore that in *rabbits the emotional stress lymphopenia does not involve necessarily* secretion by the adrenal medulla

Long Isn t *the evidence very good that adrenalin does produce* lymphopenia? Certainly in other species it produces eosinopenia and actually from Martha Vogt s experiments (24) it is a very potent stimulus to the secretion of adrenal cortical hormones as evidenced by direct measurement of those hormones in the blood Your failure to observe it in the rabbit is the one result I know of the inability to *demonstrate a stimulating effect of adrenalin on ACTH secretion*

Harris Yes

Gellhorn Isn t it partly due to the fact that in your experiments adrenalin has been injected intravenously? I believe Dr Long pointed out that adrenalin has to act for a longer period of time in order to be *effective I should also like to call attention to the fact that in two* papers we published some years ago (25 26) we showed that in the rat under a number of conditions of stress demedullation of the adrenal abolished the stress effects as indicated by lymphopenia those same results were confirmed by Dr Long and his collaborators more *recently In addition it was shown that certain conditions may result* in lymphopenia in the normal as well as in the adreno demedullated animal indicating therefore that in addition to the sympathetico adrenal discharge *there is another factor operating at the same time* That also I think is confirmed by observations of Long and others *in experiments in* which not only demedullation was carried out but the sympathetico adrenal system was eliminated either by hypothalamic lesions or spinal transection I believe that from these data it follows that we have two mechanisms

Verworn (27) has pointed out that one should speak of a number of causes rather than of one factor being the provocative agent of a given physiologic process The important question is what is the relationship between this sympathetico adrenal discharge as I would call it and the extra adrenal factor? To my mind it is *a matter of sensitivity of the two mechanisms* If you eliminate the first you have of course the second remaining Normally we would expect that the

Thorn I feel that every statement in which adrenal activation is measured only by an eosinophil fall needs qualification

Hechter Dr Thorn do you have a similar situation with respect to lymphocytes?

Thorn Unfortunately the lymphocyte changes in man are often quantitatively indeterminate as an accurate basis of measuring adrenal activation

White I do not agree completely with this statement regarding lymphocytes

Thorn That is what occurs in the human Dr White I feel that the insensitivity of the lymphocyte level in the blood in man following adrenal activation is most likely due to the fact that the feedback of lymphocytes into the bloodstream can keep pace with their destruction In other words the reservoir is relatively large whereas in the case of the eosinophils the reservoir is small and therefore changes in blood level occur more critically

White I still question this In our limited experience administration of adrenal cortical steroids in the human produces a significant lymphopenia (5) It is true that the change in absolute numbers of circulating lymphocytes is not as striking as in mice rats or rabbits but this is due to the fact that in these species one starts with a considerably higher number of lymphocytes than is the case in man I should be interested to know whether epinephrine produces a lymphopenia in a bilaterally adrenalectomized animal or in an Addisonian Or do you feel that you cannot study this in man because the initial level of lymphocytes is too low?

Thorn I did not say that the 11 oxysteroids do not cause a lymphopenia I only stated that if you are using ACTH specifically to test adrenal activation the change in circulating lymphocyte level is relatively small and unreliable whereas the fall in eosinophil level is striking and reliable

White May I ask in this connection to what degree the so called epinephrine fastness which has been described may be significant in your attempts to produce evidence of ACTH secretion with round the clock administration of epinephrine? Also what do we know about the limitations of the capacity of an individual s pituitary to produce ACTH? How soon can you run out of hormone with round the clock epinephrine?

Thorn The patients who have shown a significant quantitative increase in 17 ketosteroids in the urine following round the-clock intravenous epinephrine injections have been Cushing s patients This suggested to us that if the end organ response was quantitatively great enough then the adrenal activation following epinephrine was great

of the adrenal cortex The evidence indicates that adrenalin under stress conditions most likely reacts back on some hypothalamic mechanism rather than directly on anterior pituitary tissue, and is perhaps one factor in the increased secretion of ACTH under conditions of stress

Pincus Didn t Dr Hume show that stalk section in the dog did not lead to atrophy of the adrenal?

Harris The last time I spoke with him he was not entirely happy about his results and was talking of repeating stalk section on more dogs Perhaps Dr Thorn knows whether he has

Thorn Unfortunately Dr Hume has been out of the city for the past two or three weeks and I have not had an opportunity to discuss this point with him

Long May I say Dr Harris that if we could agree on two premises there is room for reconciliation of these various views First of all Dr Gellhorn speaks of activity of the sympathetico adrenal system as an essential part of this mechanism The activity of the sympathetico adrenal system involves more than the liberation of epinephrine Presumably it involves the activity of the head ganglion in the hypothalamus about which you are speaking That as we know is reinforced by epinephrine

The second point is on the nature of the neurohumoral transmitter If we were to agree that this was an adrenergic transmitter and epinephrine an adrenergic transmitter then there would seem to me to be reasonable ground for reconciliation of what at present, appears to be a difference in opinion

Thorn We are troubled in our studies on man by the relatively poor adrenal activation which was obtained with epinephrine administered round the clock intravenously Why is it that if epinephrine has a marked adrenal cortical stimulating effect we do not see an appreciable rise in 17 ketosteroids in the urine under these circumstances

Long Is there any reasonable doubt that epinephrine rapidly activates the secretion of ACTH?

Thorn I am worried when we say that an eosinopenia developing during hypothalamic stimulation is necessarily proof that the adrenal has been activated We have observed in certain patients with Addison s disease and more recently in patients with complete bilateral adrenalectomy that epinephrine may cause a marked fall in eosinophils Therefore I feel that there may be a direct effect of epinephrine independent of the adrenal cortex on circulating eosinophils and I do not feel that one can justifiably say that an immediate fall in eosinophils with hypothalamic stimulation necessarily means adrenal activation

Long If we accept that then there is no point in discussing Dr Hume s experiments because Dr Hume s evidence is based entirely on whether or not eosinophils fall

Thorn We obtained nothing that way We have all felt that epinephrine must potentially set off this mechanism but we have been disappointed in man with our inability to get substantial evidence of adrenal activation except *under extreme circumstances*

Fremont Smith I should like to ask Dr Harris whether a complicating role is played by the anterior lobe cells which are clustered around the tuber cinereum higher up and therefore are still left after the section has been made Long ago when I used to study that area I was always impressed with the fact that the anterior lobe really goes far up to the tuber cinereum and that you cannot eliminate it by removing the pituitary nor does any section of the stalk ever fail to leave intact a group of cells

Harris I am not sure about the answer here From the fact that so called hypophysectomized animals do not maintain the integrity of their target endocrine glands one supposes that there is not sufficient stimulus to keep them going One suggestion rather a teleological one is that this part of the pituitary has no endocrine function but is a structure laid down in order to establish the vascular connection between the tuber cinereum and the main secreting part of the gland below It is difficult *to find support for this sort of idea so I don t know if it will lead to a* profitable discussion

Fremont Smith I think your first answer is a good one Of course even if there are cells left there they are not able to keep the target organs going

Harris That is true

Long I am not clear Dr Thorn as to whether or not you are pointing *out that epinephrine does not have an effect on ACTH secretion in* the human You say if you give it continuously that you fail to find any significant rise in ketosteroids and I know it has been reported that it is relatively ineffective in the relief of rheumatoid arthritis Are you going to make the point that it is ineffective so far as ACTH secretion is concerned?

Thorn I have the feeling with of course no evidence that epinephrine *initiates an ACTH liberation but then instead of acting as a* continuous stimulator of ACTH production the action must be offset by some blocking mechanism I cannot explain that in any way except to point out that the highly stimulated individual with continuous epinephrine infusion fails to show the hormonal response which all other individuals do under stress of other types

Sprague Didn t you once find Dr Thorn that patients with rheumatoid arthritis showed slight but significant clinical *improvement in re*sponse to repeated injections of epinephrine?

Thorn Yes those were repeated intermittent injections of epinephrine

enough to be measured by the gross overall measurement We felt that the epinephrine was being administered at tolerance level since the side effects of tremor and tachycardia were present most of the time

Pincus I may complement your remarks by saying that in other types of stress, for example exposure to cold in the human, you get a very definite outpouring of 17 ketosteroids

White *In the same individual in whom epinephrine is ineffective?*

Pincus That I don t know I merely say that there are other stresses applied to the human subject which indicate that the pituitary adrenal mechanism can be activated at least to the extent that Dr Thorn wants it to be

Thorn We were discussing doses of ACTH in the order of magnitude of 10 mg Ten mg of ACTH given over an eight hour period intravenously *produces a tremendous activation of the adrenal*

Fremont Smith Morris Bender (28) found that when monkeys were frightened, the denervated facial muscles went into contraction He proved that there was a release of acetylcholine and a parasympathetic contracoup Two things which he showed are quite important and may have application to your continuous adrenalin injection One is that if you give a sympathetic stimulus you get a parasympathetic contracoup and vice versa and second that there is a species difference in that the cat is more active in its sympathetic contracoup to initial parasympathetic stimulus than the monkey Thus a parasympathetic stimulus in the cat is followed by a strong sympathetic response while a stimulus to *the sympathetic evokes only a weak parasympathetic reaction In the* monkey on the contrary there is a strong parasympathetic contracoup to sympathetic stimulations but only weak sympathetic reaction to an initial parasympathetic stimulus

In man the response is similar to that in the monkey This helps to explain why an initial sympathetic stimulus for example fear or pain evokes such characteristically parasympathetic activity as sweating vomiting diarrhea etc Bender pointed out that perhaps the reason Dr Cannon found predominantly sympathetic response to emotional stimuli was because he was working almost entirely with the cat

It occurred to me that Bender s findings might apply here that you may be *getting such a continuous parasympathetic response to your con*tinuous adrenalin injection that it may counteract the characteristic adrenalin response

Thorn I suppose that such a reaction might counteract the effect of epinephrine However of course before we tried the continuous twenty four hour infusion we tried intermittent administration of epinephrine subcutaneously over a twenty four hour period

Fremont Smith And you got nothing?

larger doses of epinephrine often caused eosinopenia in patients with Addison s disease or hypopituitarism

Selye They were untreated Addisonians at the time of the test?

Thorn That is right Since these same patients failed to respond to ACTH we assume that the epinephrine was not discharging ACTH as *the means of inducing* an eosinopenia in the Addisonian patient

Pincus If you take normal individuals that is not adrenalectomized and give them epinephrine I gather there is not much of an increase in urinary ketosteroids

Thorn No

Pincus Suppose you give ACTH at the same time?

Thorn I do not know We have never carried out the experiment exactly that way

Pincus You should do it

Harris Dr Long have you tried the effects of repeated prolonged injections of adrenalin on rats with anterior chamber grafts? Does that have any effect on the adrenal weight?

Long No we have not done that experiment Dr Harris You can reflexively produce a discharge of ACTH If you inject a few drops of hypertonic sodium chloride under the skin—it is a very painful procedure—you will observe an eosinopenia As far as our experiments go it would appear that you can produce a release of ACTH from these grafts by a painful stimulus I should like to know if you have any ideas as to the nature of this transmitter It seems to me this is a key point Have we one transmitter for six hormones or have we to think of six transmitters if all the pituitary hormones are regulated in this way? You suggested such a mechanism possibly regulated the secretion of the lactogenic the thyrotropic ACTH and the gonadotropic hormones What type of neurohumoral link can you think of one neurohumoral transmitter or six?

Harris That is a question that can be answered at the moment only by asking further questions One is how many hormones are liberated by the pituitary? Are six individual chemical substances liberated individually into the blood stream? Second it might be that the same humoral transmitter activates different substances according to the amounts transmitted Possibly different hormones have a different threshold for the same transmitter I don t know this is pure speculation of course

There are only two bits of evidence The first is from the work of Markee and his colleagues at Duke University (29) who suggest that there is an adrenergic substance transported to the pituitary to activate gonadotropic release The other fragment of evidence which may have no bearing on the point at all is the fact that some substance with

Sprague Was not the response enough to make you think that their adrenals were stimulated?

Thorn I am certain their adrenals were stimulated but of course I cannot be certain our treatment was the mechanism responsible for adrenal stimulation although quite probably it was

Long The long continued administration of epinephrine does enlarge the adrenals at least in animals

Thorn I do hope that these differences in observations between animal experiments and man can be reconciled In our experience it was only when we stimulated a patient with Cushing s disease with intravenously administered epinephrine that we were able to get an appreciable rise in the excretion of 17 ketosteroids and 11 oxysteroids

Long Dr Harris mentioned the grafts of the anterior pituitary into the eye that we have carried out and that Dr Fortier also has done * I think it should also be called to your attention that in both laboratories we have found that the direct application of epinephrine to those grafts does result in discharge of ACTH as measured by an eosinopenia I believe that Dr Fortier also found that the direct application of histamine to the grafts provoked a similar response But those two substances were the only ones that did so of a number he tried

Selye Actually Dr Fortier did not try very many substances but among the ones that he did try histamine and adrenalin were as a matter of fact the only two that were effective under these conditions However he does not feel that this means that only these two substances can stimulate the pituitary by local application

I think one should also consider the possibility that any or at least a large number of the agents which change the normal homeostasis of pituitary cells might cause a discharge of ACTH in response to local injury of some sort in the pituitary cells just as histamine is liberated from practically all cells under the influence of almost any local damaging agent

I should like to ask Dr Thorn whether he feels that the positive eosinopenic response in Addisonians which he has obtained after epinephrine administration is dependent on though not mediated through the presence in the body of corticoids Those patients were presumably treated with corticoids at the same time

Thorn No we have had patients who have displayed an eosinopenia with epinephrine who were not being maintained on cortisone In our original observations on epinephrine we pointed out that it was necessary to use a small dose of epinephrine namely 0 3 mg in order to obtain a physiological response but even in our earlier experiments

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FORTIER C Verbal comm at

Li I don t think your question is pertinent here For one thing I don t understand what you mean when you talk about positive charge At any rate the hormone as it functions inside the gland is very different from the hormone once it has been extracted from the gland There must certainly occur during the process of extraction changes in the molecule and in the tissues For this reason it is difficult to explain phenomena occurring *in vivo* in terms of evidence which was obtained *in vitro*

Long Dr Harris has asked a provocative question Are there six anterior lobe hormones or only one?

Li Biological studies indicate that there are six separate biologic functions of the gland Whether they are due *in vivo* to one molecule or six molecules is something we cannot answer until we are able to isolate from the blood the hormone responsible for these functions

Harris Apart from the possibility of isolating definite entities from the blood is there a possibility of seeing how many substances are secreted by injecting for example estrogens which produces atrophy of the ovaries? Does that also result in the atrophy of the adrenal cortex or the thyroid? Or can you by injecting thyroxin induce specific athropy of the thyroid by injecting estrogen specific atrophy of the ovaries?

Li Yes you can identify all these biological actions

Harris From that type of experiment are there again six different substances secreted?

Li Or again we should say six different activities

Pincus I think Dr Ingle has studied the estrogen problem If I remember some of his experiments there was adrenal hypertrophy following estrogen is that correct?

Ingle Yes the adrenal cortex hypertrophies but the gonads atrophy

Thorn Does the reaction depend somewhat on the dose of estrogen? There is evidence to suggest that in man the reaction of the adrenal to estrogen may be modified by the size of the dose

Ingle In the rat it depends upon the dose and upon the age of the animal There are many examples of dissociations in the effects of anterior pituitary hormones upon their target organ which make necessary the assumption that the secretion of one anterior lobe hormone can be increased while the secretion of another is suppressed Dr Selye has written about this at some length (30) The anterior pituitary in meeting the needs of the organism for more adrenal cortical hormones releases *more ACTH and simultaneously decreases its* production of growth and gonadotropic hormones

Long Dr Sayers you have been charged with the authorship of another hypothesis Do you want to comment?

Sayers I should like to express my admiration for the beautiful experiments of Dr Harris

properties rather like histamine is present in the tuber cinereum and in the different lobes of the pituitary gland It was found in rather greater concentrations as far as we could see in the region of the tuber cinereum where the portal vessels take their origin It has not been definitely established that it is histamine but it has properties like histamine *

Long What are the possibilities for neurohumoral transmitters? We have adrenalin choline and possibly an unknown group

Selye Histaminergic

Loewi Some people speak about histamine as urger For instance Dale discussed the possibility that afferent nerves might transmit impulses by releasing histamine On the other hand it was found long ago that histamine stimulates the adrenal medulla Why then should not histamine act by stimulating the adrenal medulla? Furthermore what is your answer to Dr Long s question as to the possibility that your transmitter might be of adrenergic character?

Harris I think that is a definite possibility

Loewi If this possibility would prove correct we would have to deal with only one transmitter

Hechter As a result of recent studies on the chemistry of ACTH by Dr Astwood and Dr Morris in England it would appear that ACTH is a positively charged substance which can be absorbed rather firmly on protein or on complex polypeptides Dr Astwood in reviewing the various methods utilized for extracting ACTH from the pituitary at the 1951 Laurentian Hormone Conference commented on the fact that ACTH appears to stick very firmly to the pituitary tissue If this be so then you are faced with a rather curious situation in that ACTH a substance which appears to stick to the pituitary tissue, must be discharged within a matter of seconds in response to stress through the mediation of one or more transmitters This would appear to raise a basic biochemical question in that the transmitter must instantaneously release ACTH from its firm attachment to pituitary tissue

Long Well it sticks on denatured protein We don t know what its stickiness is on the native protein as it exists in the cell

Hechter If it sticks on denatured protein and it sticks on polypeptides why can t it stick on native protein?

Long I don t know why it shouldn t

Li I don t know what you mean by sticking

Hechter I am speaking of the thesis presented by Morris and Astwood that ACTH is a small molecule which can stick or associate itself or be absorbed on protein or polypeptide or on other types of acidic material

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Harris G W Jacobsohn D and Kahlson G Unpublished work

Sayers The possibility exists that they had a deficiency of TSH

Harris Yes that was one of the possibilities

Sayers I presume that you have had that in mind Dr Cheng left our laboratory before we could get at that particular problem There is one other question that I have You told us of a lesion in the anterior lobe of an animal whose response to emotional stress was abolished Do you interpret this to mean that with the lesion placed as it was there was no regeneration of hypophyseal portal vessels? The remaining hypophyseal tissue was relatively close to the median eminence

Harris In that particular animal there wasn t

Sayers You were sure of that?

Harris Yes

Sayers I was just wondering whether the excellent functional capacity of the transplant placed near the median eminence was determined by its proximity to the median eminence

Harris Many things vary the regeneration such as the cleanness of the surrounding tissues The lesion in that rabbit was an electrolytic lesion with a large mass of coagulated tissue which would act as a thick barrier to regeneration

Sayers Finally I am very much interested in the location of the lesions in the hypothalamus as related to the ability of the animal s hypophysis to discharge ACTH I find in reading the literature discordant results regarding the location of the lesions and induction of inertia in the pituitary adrenocortical system Could you outline the difference between your experiments and those of Hume with regard to the location of the lesions? As I understand it in Hume s studies the lesions were in the anterior part of the hypothalamus

Harris I am not quite clear where Dr Hume s lesions were I don t know whether he has published his work on this in detail if so I have not yet seen it I know the lesions appeared to be more anterior but when I was talking to him he had not studied his material histologically and so I don t know whether it would really be very profitable to discuss the difference between Hume s results and ours Our lesions (Figure 17) were centrally situated in the hypothalamus and in rather the median part instead of the lateral That region as far as I know from the evidence of Ranson s experiments (5) was not the region that on stimulation gave secretion of adrenalin The region he stimulated that gave adrenalin secretion was the lateral hypothalamic region and I think also the region around the fornix From our experiments it would appear that the region of the hypothalamus important for adrenalin secretion is not coincident with the region that blocks the emotional lymphopenia But that I feel is something that wants more investigation the exact site of the hypothalamic lesions perhaps in terms of nuclear groups and fiber tracts

I am convinced that a major difficulty in the problem of pituitary regulation concerns the accuracy and specificity of the indices we employ to measure discharge of ACTH from the adenohypophysis I am inclined to think that when we have better indices available for measuring discharge of ACTH many of the discrepancies which appear to exist between laboratories will be resolved

Dose is an important factor to be considered in the evaluation of epinephrine as a regulator of pituitary ACTH activity Is the dose of epinephrine which must be injected to induce discharge of ACTH of the same order of magnitude as the amount of epinephrine which is actually discharged from the adrenal medulla during stress? I should like to hear comments from Dr Gellhorn and Dr Loewi regarding the quantity of epinephrine that is discharged from the adrenal medulla during stress and whether the doses of epinephrine which must be injected to induce discharge of ACTH are of the same order of magnitude

Regarding the point that Dr Fremont Smith brought up the possibility that when one injects epinephrine its effect is counteracted by a stimulation of the parasympathetic system I should like to point out that Dr Fortier has demonstrated that acetylcholine brings about a discharge of ACTH

Long How was that done directly on the isolated tissue or into the animal?

Selye Into the animal

Sayers The combination of epinephrine and acetylcholine (injected simultaneously) has not yet been tried Cholinergic as well as adrenergic agents stimulate the pituitary to discharge ACTH We don t know the mechanism at this time

Long A lot of other substances will do that when injected parenterally

Sayers In regard to the point that Dr Harris brought up of just ho many neurohumors are elaborated by the hypothalamus I have a bit of pure speculation It is possible that a single neurohumor sensitizes the animal s hypophysis to the various target gland hormones For example neurohumor plus change in the titer of thyroid hormone in the body fluids alters the rate of discharge of TSH neurohumor plus change in the concentration of cortical steroids alters the rate of discharge of ACTH etc Such a mechanism would avoid the necessity for six neurohumors one for each of the adrenohypophyseal hormones

It seems to me that in chronic situations there is no doubt that one or two tropins can be discharged to the exclusion of the others

In regard to the experiments with the transplants I should like to ask Dr Harris if he has given the subtemporal transplant animals thyroid hormone

Harris No

penia In a few animals both types of experiment pointed to the same thing as far as the mammillary body was concerned

Gellhorn The mammillary body has been considered to be one of the areas for regulation of sympathetic adrenal secretion I don t want to resume that discussion again It may be entirely insignificant in the rabbit

Harris May I ask what the evidence is Dr Gellhorn?

Gellhorn Well isn t that true of the work of Ranson (5) for instance? Ranson stimulated the mammillary bodies and the lateral nuclei too i e, hypothalamic nuclei

Harris I may be wrong but I was not aware that he had stimulated the mammillary bodies Did he get adrenalin secretion by stimulating the mammillary bodies?

Gellhorn We have been doing that routinely We insert the electrodes into the mammillary bodies of the hypothalamus We get maximal pupillary dilatation and retraction of the nictitating membrane In that same area if you record the blood pressure you first get an immediate rise then a decline and a second rise which is absent when you eliminate the innervation of the adrenal medulla I have not studied the area histologically but from numerous observations I would believe that this is a correct statement Moreover I do not think that one can separate sympathetic foci from foci eliciting the secretion of adrenalin The latter results from more prolonged or more intense stimulation

Harris What spread of current is there in these experiments?

Gellhorn That I could not say The stimulation involves three volts

Harris That seems rather high These were anesthetized animals?

Gellhorn Anesthetized animals

Long Have you seen the paper of Van Arman (51)? He measured the fall in the epinephrine content of the adrenal medulla of rats who had been given a small dose of insulin These experiments were done for an entirely different reason he was studying the rate of regeneration of epinephrine in the gland In the rat he found in the four hour period that covered the hypoglycemia and recovery that each adrenal lost 15 μg of epinephrine That would be a total of 30 μg of epinephrine from the adrenals of a rat weighing about 300 gm If you calculate that it is considerably higher than what has been estimated for a larger animal The degree of insulin shock was not severe because the animals all recovered spontaneously

But certainly in our experience the amounts of epinephrine that are effective in stimulating ACTH production are within the secretory range of the medulla and this is not a pharmacologic effect of epinephrine

Thorn It is interesting to consider that clinically the Cushing s picture is a rarity in patients with pheochromocytoma I believe that one

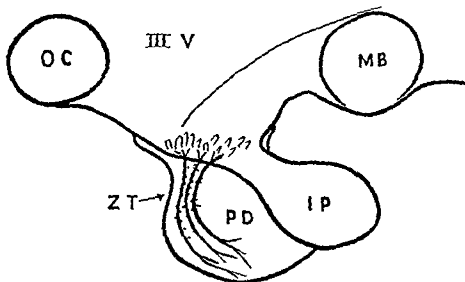


FIGURE 17 Diagram of a mid line sagittal section through the hypo thalamus and pituitary gland of a rabbit showing the sites (stippled areas) where lesions in these structures interrupted the lymphopenic response to emotional stress These sites included the mammillary bodies (M B) the posterior part of the tuber cinereum and the anterior pole of the pituitary formed by the zona tuberalis (Z T) The zona tuberalis is the part of the anterior pituitary gland which transmits the hypophyseal portal vessels I P intundibular process O C optic chiasma P D pars distalis III V third ventricle

Pincus Dr Hume recently gave a lecture in which he commented on this point His original idea that the lesions were in the paraventricular nucleus he now abandons and he says he was misled by various indications He feels that the effective lesions are essentially on the floor of the third ventricle which brings them very close if not almost exactly to where yours are

Gellhorn Dr Harris in your experiments were the mammillary bodies included or not?

Harris In some the mammillary bodies were included There was one rabbit in which more by good luck than anything else the mammillary body was completely and fairly precisely destroyed The surrounding structures were all intact And that lesion did block the emotional lymphopenia In another animal the mammillary bodies were stimulated The tip of the electrode was in the mammillary bodies (The spread of current I might mention is less than 0 5 mm) Stimulation of the mammillary bodies did produce an emotional lympho

I do not find it too difficult to suppose that there would be several neurohumors Years ago it was hard to believe that the pituitary with its limited number of secreting cell types could possibly secrete six or more hormones There is an analogy to the liver which is made up chiefly of one kind of cell but which probably carries out a hundred different reactions This makes it easier to believe that the hypothalamic region could elaborate a great many substances even though the cells there do not to the histologist look like glandular elements

Harris There is another point The clinical syndromes such as acromegaly tend to be multiglandular but I don t know how significant it is that with a tumor of one cell type there are effects indicating excess secretion of several hormones With regard to the number of transmitters it may perhaps be that one transmitter in different amounts could evoke one or another tropic hormone discharge

Astwood It is hard to imagine though Dr Harris that a substance which is going to bring about say ovulation in the rabbit would at the same time cause enlargement of the adrenal It does not make physiological sense

Harris The intact animal seems to work rather that way Coitus in the rabbit for example will produce a discharge of ACTH I believe And emotional stress will affect the discharge of many of the pituitary hormones

Pincus Did your emotionally stressed rabbits ovulate?

Harris No We looked for that once or twice but we did not see it

Thorn Would you feel that if you did not postulate several neurohumoral substances the feedback system would in essence have to work primarily on the pituitary rather than on the hypothalamus?

Harris Unless it worked at different threshold levels in the hypothalamus

Selye One ought to point out also that nervous stimuli if they are acute can cause ovulation in short term experiments Nevertheless continuous exposure to a nervous stimulus would cause ovarian atrophy at a time when it causes adrenocortical stimulation So that certainly the two do not always run parallel

I find Dr Sayers suggestion most attractive perhaps others might also comment upon it There does appear to be a possibility that there is only one neurohumoral transmitter for activation of pituitary cells by any kind of response but that a multiplicity of agents can condition the cells so as to produce one or the other type of tropic hormone in response to this neurohumoral transmitter

Harris Then it makes it difficult to see how the pituitary can be driven above the level of the peripheral blood content

Selye I am not speaking about Dr Sayers feedback theory

or two patients have been reported in the literature in which a Cushing like clinical picture was associated with a pheochromocytoma but in general hyperfunctioning adrenal medullary tumors do not induce a Cushing s picture This suggests of course that if the substance re leased by these tumors is a normal stimulant of the hypothalamic and pituitary there must be an effective shutoff mechanism

Pincus Do these people show any indications of adrenocortical atrophy?

Thorn Not those that I have seen

Sprague It may be that Cushing s syndrome is a more extreme evi dence of adrenal stimulation than one could expect

Thorn I agree with that

Sprague I know of cases of pheochromocytoma in which the excre tion of formaldehydogenic steroids was increased above normal pre sumably indicating adrenal cortical stimulation

Thorn On the other hand it takes only a week of intensive ACTH therapy in some patients to precipitate the picture of Cushing s syndrome

Sprague There is no doubt a great variation in individual response to a given amount of ACTH

I should like to ask Dr Harris a question Heinbecker (32) has described destructive lesions in the paraventricular nuclei of the hypo thalamus of patients with Cushing s syndrome The presumption is that such lesions give rise in some manner to chronic overproduction of adrenotropin Have you ever found any evidence that a destructive lesion anywhere in the hypothalamus gives rise to chronic hypersecre tion of adrenotropin?

Harris No we have not but I think our experiments were too short for there to have been any possibility of observing such a thing

Astwood May I reopen the question of how many transmitters there must be Dr Harris question about how many pituitary hormones there are has already been answered Further to that I think one could marshal good evidence to show that each one of at least six hormones can be secreted independently of the others under physiological condi tions In certain instances some of which have already been mentioned the conditioning factor is not a drop in the circulating target gland hormone For example in the regulation of the estrous cycle where three pituitary hormones are involved it is difficult to envision that the pituitary is regulated entirely by the concentration of circulating estro gen and progesterone It is possible also that there may be one or more additional pituitary hormones aside from the six well recognized ones If each one can be independently secreted one would have to suppose that there would be a corresponding neurohumor to evoke the appropri ate secretion from the anterior lobe

White I was speaking of using the stalk sectioned animal in which repair of vessels had been prevented such as by your paper strip as an assay animal for various materials which are suspected to be the neurohumors or for extracts from the region in which you believe the neurohumors to be elaborated

Harris Where would you inject though?

White That is why I asked the question as to whether epinephrine injected systemically in your preparation would give you evidence of ACTH release

Harris Then you would inject systemically?

White If epinephrine had no effect under these circumstances then you would have an assay preparation

Long Isn t that Dr Hume s hypothesis?

Thorn Yes

Long In effect Dr Hume said this material comes from certain hypothalamic areas If it is injected into the animal the pituitary would not respond if that material had been removed by certain hypothalamic lesions

Thorn Hume obtains a good eosinopenic response under these circumstances but it has not been possible up to now for him to carry the reaction one step further namely to demonstrate decisively adrenal activation as the cause of the eosinopenia by measuring urinary 17 ketosteroid or 11 oxysteroid output

White That is why I asked the question about the nonspecific agents whether the only pathway for this material was via the portal hypophyseal vessels or whether this material *entering some other vascular pathway* from the hypothalamus would also activate the pituitary If it *does then the stalk section idea which I suggested is not valid* but if it does not then it seems to me there is beginning to be set up a bioassay for *studying what some of these neurohumors might be*

Harris Dr White would you explain the difference between your stalk cut preparation and the pituitary tissue transplanted to a distal site where it is known to be inactive also?

White I should have difficulty explaining it except for the suggestion of Dr Selye with which I agree in part namely that an agent acting directly on tissue—he specifically mentioned histamine for example—might cause a release of intracellular material as a result of tissue damage rather than as a result of secretory activity of the cells

Harris How is that different though if the material is injected in the ear?

Pincus There is no difference if you inject the material systemically

White There is no difference It is a question of dilution You might apply a good deal more material locally than you would systemically

Harris Wouldn t the same thing hold?

Sayers As I say it is pure speculation There is no evidence to support it

White It seems to me there is much speculation about neurohumors from the hypothalamus how they work and what they are At one of the previous Conferences I asked whether anyone could suggest a method for bioassay of such substances so that attempts to purify them chemically could proceed I think at that time there was no response I wonder, however whether your stalk sectioned animal with the paper in place so that the portal vessels do not regenerate is an animal in which such assays could be conducted? The answer to that question would be more or less dependent upon whether such an animal responds to nonspecific stresses with a change in indices which we attribute to ACTH release Have you studied other stimuli than the emotional any of the so called nonspecific substances which will cause ACTH release normally? Do these stresses cause ACTH release in your stalk sectioned animal in which regeneration of the vessels is prevented?

Harris No we have not studied that directly What we are doing at the moment—in collaboration with Dr Curt von Euler—is making pituitary transplants to a site remote from the hypothalamus and then testing various substances looking not for ACTH release but for ovulation which we think is perhaps a more discrete response

White You are picking substances more or less at random which will cause the transplanted pituitary to secrete gonadotropin?

Harris Yes

White If one took the animal without the transplanted pituitary prepared in precisely the same way with the stalk section and if one administered to that animal let us say blood from the portal hypophyseal vessels does that animal then ovulate?

Harris That would be very nice to do but technically very difficult

White The securing of the blood?

Harris The securing of the blood yes

White And I suppose extracts of the relatively ill defined region *which you think secretes this material would also be difficult to obtain*

Harris That is one of the things we have in mind now on transplanted tissue It seems much easier to apply extracts to pituitary tissue transplanted say under the skin of the ear rather than to the gland in its normal situation but with the stalk cut

White Except that there you have the factor which Dr Selye mentioned namely the effects of irritating materials on cellular reactivity and cellular fragility

Harris You had in mind injecting intravascularly into the blood supply of the stalk-cut gland?

paper plates in between the stalk that you are not at the same time devascularizing large areas of the pituitary? I think you mentioned that a large part of the blood supply of the anterior lobe normally was supplied through this portal system Consequently when you interrupt that is there not a possibility that you have an ischemia or a relative degree of ischemia of the anterior lobe?

Harris Figure 18 is an animal with a plate between the cut ends of the stalk It is an India ink injected preparation From the amount of ink in the anterior pituitary one would gather that the vascular tree compared with the brain is fairly great There is a difference from the normal gland though in that the part of the pituitary which normally is most injected with ink is the anterior pole where the portal vessels come in but in this type of case the anterior pole is less injected than the more posterior regions Thus there is that difference when the stalk is cut But the vascular tree anyway is certainly rich in the stalk sectioned animals

Astwood Why is it Dr Harris that in the experience of Greep and also in our laboratory with sectioning of the stalk by the method that Greep used there is infarction of the central portion of the anterior lobe?

Harris That always happens in the rat Whichever technique is used for stalk section there is an area of central fibrosis It is difficult to see after a long time but it is always there within the first week or two However I think that cannot be the explanation as to why after stalk section and the insertion of a plate the pituitary tissue does not function There is not a correlation between the amount of tissue remaining or the vascularity of the tissue and the return of the function There is of course the possibility that although the vascular tree is rich the rate of blood flow is reduced That is also a possibility with regard to the graft experiments that the rate of blood flow in the vessels of the temporal lobe grafts is less than in the vessels of the hypothalamus grafts although the control grafts appear to be as richly vascularized as the grafts under the hypothalamus What possibility there is for getting any data on the rate of blood flow in the graft vessels I don t know Technically it would be very difficult

Long Is the histological appearance normal? It would not be possible that you had an infarct immediately after operation and then ultimately the pituitary did not regenerate but the blood vessels grew back in and around perhaps giving an indication of the viability or the functional activity of those areas? Are the sections from those anterior lobes changed in any way after such an operation?

Harris The cytology of the pituitary in the stalk-cut rats was not investigated but the cytology of the pituitary tissue in the rats with grafts seemed quite clear The cytology of the grafts under the median

Thorn I don t think we are clear as to how we apply your substances

Harris We have in mind two routes one under the skin of the ear so the transplant is in a pool of the substance and the other is into the arterial supply of the ear, thus perfusing the transplant by its vessels

Ingle Years ago we transplanted the anterior pituitary (autogenously) to various parts of the body We had our best success with transplants under the capsule of the ovary There was only partial maintenance of the adrenal cortex and the gonads but our results were somewhat better than with grafts to the anterior chamber of the eye

Long Dr Harris do you know that in his book on the pituitary body Dr Harvey Cushing in 1912 said that the brain was the best place to transplant the anterior lobe of the pituitary?

Harris I believe he was the first person to transplant the pituitary

Long May I ask if you are entirely satisfied when you place these



FIGURE 18 Microphotograph of a midline sagittal section through the hypothalamus pituitary gland and base of skull of a rat with the vascular system perfused with India ink The pituitary stalk had been sectioned and a paper plate (P) inserted at the site of section Note the plate (4 mm × 2 mm) interrupts any pathway for regeneration of vessels between the primary plexus in the median eminence (M E) and the anterior pole of the pituitary gland The anterior pole of the gland in this rat appears relatively avascular compared with the main bulk of the pars distalis (P D) which is so well vascularized that it appears almost black in a section this thickness This animal remained anoestrus for 79 days after operation when it was killed Section 300 μ thick unstained Reprinted by permission from Harris G W Oestrous rhythm Pseudopregnancy and the pituitary stalk in the rat *J Physiol* 111 347 (1950)

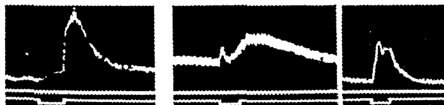


FIGURE 20 Stimulation of hypothalamus (coil at 6 cm for 10 sec) before (left) during (center) and after perfusion (right) with adrenalin 1 200 000 (3 ml perfused in 3 5 min) Designations as in Figure 19 Reprinted by permission from Darrow C W and Gellhorn E Effects of adrenalin on reflex excitability of autonomic nervous system *Am J Physiol* 127 243 (1939)

Another indicator that has been used is the nictitating membrane On stimulation of a peripheral nerve or of the hypothalamus the nictitating membrane a sympathetic indicator contracts But after injection of adrenalin the response of the nictitating membrane to reflex or hypothalamic stimulation may be greatly reduced although the nictitating membrane itself is not influenced by the small amounts of adrenalin used in these experiments In other words a slight amount of adrenalin diminishes the reactivity of the hypothalamus very considerably and this factor may be potent in the human who after all is not under the influence of any anesthetics I should like to emphasize this fact because most of the work reported here was done on anesthetized animals under conditions of severe stress

Pincus I hesitate to introduce a possibly complicating factor but I should like to remark that in a number of studies that Dr Hechter and Mr Hopkins and I have conducted we have evidence for an ACTH inactivating factor in the blood * We found it in the blood of the rat the human and in beef blood as well This factor appears to differ somewhat in its concentration in the blood of different species It is rather low in concentration in beef blood as compared to human blood for example

One of the speculations that has occupied our attention although we have not done any experimental testing is the possibility that this factor might also act as a regulatory factor in the peripheral sense rather than at the level which you are discussing It might very well affect the level of circulating steroids Whether it would have any effect on any other fraction of this mechanism I don t know But it might at least in part be responsible for some of the anomalous pictures that we have had

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* PINCUS G HECHTER O and HOPKINS T Unpublished data

eminence appeared normal with chromophil and chromophobe cells but this was not the case with the temporal lobe grafts

Gellhorn May I illustrate with Figures 19 and 20 a problem which came up a little earlier in which Dr Thorn called attention to the fact that the effect of adrenalin in the human is quite different from that in animals? It may have to do with the following phenomenon

The phenomenon was first described by Hoskins (33), and then some years later Dr Darrow and I (34 35) carried out similar observations on the effect of adrenalin on the activity of the sympathetic centers both under conditions of reflex and hypothalamic stimulation

If under control conditions you stimulate the sciatic and get a slight pressor response you may find on repetition of this experiment after you have given a small amount of adrenalin which elevates the blood pressure level no pressor response or you may actually get a slight de crease in blood pressure Such experiments were made by Hoskins if I remember correctly

Similar results are obtained with stimulation of the hypothalamus with the blood pressure as an indicator of the excitability of this au tonomic center

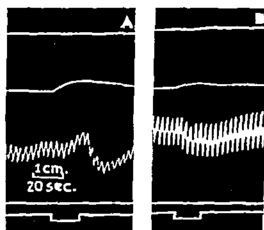


FIGURE 19 Influence of adrenalin on the reflex excitability of the autonomic system The effect of stimulation of the brachial plexus (coil at 5 cm for 15 sec) before (A) and after (B) perfusion with adrenalin 1 200 000 3 ml per min for 3 min Record from above downward sympathectomized nictitating membrane (N M) normal N M blood pressure signal indicating stimulation of brachial plexus Reprinted by permission from Darrow C W and Gellhorn E Effects of adrenalin on reflex excitability of autonomic nervous system *Am J Physiol* 127, 243 (1939)

In the case of the one you sent us we tried to do it in human blood but we did not have enough What blood did you use?

Li Rat

Pincus Rat blood is quite potent

REFERENCES

1 Harris G W Blood vessels of rabbit s pituitary gland and significance of pars and zona tuberalis *J Anat* 81 *343 (1947)*

2 Colfer H F de Groot J and Harris G W Pituitary gland and blood lymphocytes *J Physiol* 111 *328 (1950)*

3 Dougherty T F and White A Influence of hormones on lymphoid tissue structure and function Role of pituitary adrenotrophic hormone in regulation of lymphocytes and other cellular elements of blood *Endocrinology 35, 1 (1944)*

4 de Groot J and Harris G W Hypothalamic control of anterior pituitary gland and blood lymphocytes *J Physiol* 111 *335 (1950)*

5 Magoun H W Ranson S W and Hetherington A Liberation of adrenin and sympathin induced by stimulation of hypothalamus *Am J Physiol* 119 *615 (1937)*

6 Popa G T and Fielding U Portal circulation from pituitary to hypothalamic region *J Anat* 65 *88 (1930)*

7 Wislocki G B and King L S Permeability of hypophysis and hypothalamus to vital dyes with study of hypophyseal vascular supply *Am J Anat* 58 *421 (1936)*

8 Green J D and Harris G W Neurovascular link between neurohypophysis and adenohypophysis *J Endocrinol* 5 *136 (1947)*

9 Green J D Comparative anatomy of hypophysis with special reference to its blood supply and innervation *Am J Anat* 88 *225 (1951)*

10 Green J D and Harris G W Observation of hypophysio portal vessels of living rat *J Physiol* 108 *359 (1949)*

11 Dempsey E W and Uotila U U Effect of pituitary stalk section upon reproductive phenomena in female rats *Endocrinology* 27 *573 (1940)*

12 Dempsey E W and Searles H F Environmental modification of certain endocrine phenomena *Endocrinology* 32 *119 (1943)*

13 Westman A and Jacobsohn D Endokrinologische Untersuchungen an Ratten mit durchtrenntem Hypophysenstiel Reaktion der Ovarien auf Prolanzufuhr *Acta obstet et gynec Scandinav* 18 *109 (1938)*

14 Greep R O and Barrnett R J Effect of pituitary stalk section on reproductive organs of female rats *Endocrinology* 49, *172 (1951)*

15 Cheng C P *et al* Discharge of adrenocorticotropic hormone in absence of neural connections between pituitary and hypothalamus *Am J Physiol* 158 *45 (1949)*

16 Barrnett R J and Greep R O Pituitary gonadotropic activity of stalk sectioned male rats *Endocrinology* 49 *337 (1951)*

particularly if it would vary under certain of the experimental conditions that we have tried

Harris Have you noted any variations in the anti ACTH substance under different conditions?

Pincus We have examined it in blood taken at various times from animals but have found no great variation We have not investigated it systematically because we have been considering the possibility that it may be a system involving a co factor as well as a heat labile substance, in the blood But the one experiment that we have done recently which suggests that we may have a basis in this mechanism is the fact that the *blood of the hypophysectomized rat is quite active even though the* pituitary is missing I am not quite sure what this means but at least in that type of endocrine state the inactivating factor was still present

Li Dr Pincus may I ask what ACTH preparation you used to demonstrate this anti factor?

Pincus We have used four preparations One was a pituitary hydrolysate prepared by the Armour Company which is very rapidly inactivated The second one was an extract of whale pituitary in fact two types of extract one a so called protein extract and the other a hydrolysate which are also inactivated The third is a preparation made by Dr Li which is not inactivated in beef blood at all The fourth is a preparation made by Dr Astwood which is also not inactivated in beef blood at all On the other hand Dr Astwood s preparation is inactivated to a much lesser extent in human blood than the Armour preparation Our explanation for the difference is that the Armour preparation and perhaps the whale preparation introduced this co factor into the beef blood whereas in the human blood the co factor is not necessary

Li The reason I raise this question is that it has been demonstrated by Dr Smith (36) in Utah that some preparations contain a proteolytic activity In our own experiments we always boil the ACTH solution during certain stages of preparation which might destroy this proteolytic enzyme activity

We have also tried to find out whether the plasma or blood of animals would inactivate ACTH So far we have always found that no inactivation occurs Thus I am wondering whether this factor which you observe in the blood might be due to the enzyme contaminant in your preparation if it has not been treated with heat or acid as we do

Pincus The Armour preparation we used was an acid hydrolysate How thorough a one it was I don t know But I think the most important thing is that we have heated this blood which inactivates the preparation at 50 degrees C for an hour and it no longer inactivates We recover 100 per cent of the activity under those conditions whereas if the blood is not heated, we recover 15 to 20 per cent of the activity

DETERMINATION OF ADRENAL CORTICAL STEROIDS IN BLOOD

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AN INVESTIGATOR WHO sets out to determine the quantities of adrenal steroids in blood first has to answer a very important question and that is just what compounds is he going to measure? Therefore I should like to discuss briefly the evidence for the compounds which we might expect to find in blood

Dr Samuels Dr Reich and I have isolated 17 hydroxycorticosterone from the adrenal venous blood of dogs and with the help of Dr Zaffaroni corticosterone has also been isolated from similar material (1 2) Dr Gassner working with our group and with the group at Armour & Co has succeeded in isolating another compound or compounds which has androgenic activity from the adrenal venous blood of cattle We found in the adrenal venous blood of dogs a compound which is more polar than compound F (3) and which has not as yet been identified It has 240 millimicra adsorption which is characteristic of the alpha beta unsaturation and it elutes from the column after compound F Possibly the same compound has been isolated from incubations of adrenal homogenates by Hains as he has reported finding a more polar compound than F We have also isolated from the peripheral blood of human subjects after the administration of ACTH a compound which appears to be F It would thus appear that 17 hydroxycorticosterone is the chief compound secreted by the adrenal gland which has the hydroxl group in the 17 position and that corticosterone is the chief compound secreted which does not have this group However we must keep in mind that there is a compound or compounds secreted which has or have androgenic activity and there is also a more polar compound which may be related to Dr Kendall s amorphous fraction Considering the techniques which have been used thus far there may be in addition small quantities of other compounds present

Kendall Does the amount of ACTH administered make a difference that is are the compounds released from the adrenal cortex after a small

17 HARRIS G W Oestrous rhythm Pseudopregnancy and pituitary stalk in rat *J Physiol* 111, *347 (1950)*
18 CHENG C P *et al* Discharge of adrenocorticotropic hormone from transplanted pituitary tissue *Am J Physiol* 159, *426 (1949)*
19 MCDERMOTT W V *et al* Release of adrenocorticotropic hormone by direct application of epinephrine to pituitary grafts *Proc Soc Exper Biol & Med* 73, *609 (1950)*
20 HARRIS G W The secretomotor innervation and actions of the neurohypophysis and investigation using the method of remote control stimulation Thesis for M D degree Cambridge University Cambridge England 1944
21 HARRIS G W and JACOBSOHN D Functional grafts of the anterior pituitary gland *Proc Roy Soc B* (In press)
22 FISHER C INGRAM W R and RANSON S W *Diabetes Insipidus and the Neuro Hormonal Control of Water Balance* Ann Arbor Mich Edward Bros Inc 1938
23 MAGOUN H W FISHER C and RANSON S W Neurohypophysis and water exchange in monkey *Endocrinology* 25, *161 (1939)*
24 VOGT M Observations on some conditions affecting rate of hormone output by suprarenal cortex *J Physiol* 103, *317 (1944)*
25 GELLHORN E and FRANK S Sensitivity of the lymphopenic reaction to adrenalin *Proc Soc Exper Biol & Med* 69, *426 (1948)*
26 GELLHORN E and FRANK S Lymphopenia and the secretion of adrenalin *Proc Soc Exper Biol & Med* 71, *112 (1949)*
27 VERWORN M *Kausale und konditionale Weltanschauung 2nd ed* Jena G Fischer 1918
28 WEINSTEIN E A and BENDER M B Pupillodilator reactions to sciatic and diencephalic stimulation *J Neurophysiol* 4 *44 (1941)*
29 MARKEE J E SAWYER C H and HOLLINSHEAD W H Adrenergic control of the release of luteinizing hormone from the hypophysis of the rabbit *Recent Progr Hormone Research* 2 *117 (1948)*
30 SELYE H *Stress* Montreal Acta Inc 1950
31 VAN ARMAN C G Amino acids and amines as precursors of epinephrine *Am J Physiol* 164, *476 (1951)*
32 HEINBECKER P Pathogenesis of Cushing s syndrome *Medicine* 23, *225 (1944)*
33 HOSKINS R G and ROWLEY W N Effects of epinephrine infusion on vasomotor irritability *Am J Physiol* 37, *371 (1915)*
34 DARROW C W and GELLHORN E Effects of adrenalin on reflex excitability of autonomic nervous system *Am J Physiol* 127, *243 (1939)*
35 GELLHORN E Internal environment and behavior influence of variations in blood sugar on functions of brain *Am J Psychiat* 97, *1204 (1941)*
36 ADAMS E and SMITH E L Proteolytic activity of pituitary extracts *J Biol Chem* 191 *651 (1951)*

White You haven't done recoveries?

Nelson On the adrenal venous blood we have spent some time studying recoveries of such compounds as cortisone desoxycorticosterone and compound F We can extract most of it from the blood but the problem is in obtaining blood with high enough levels so that a large quantity of steroid can be gotten out

Ralli How much blood do you use? As much as you can get I suppose

Nelson As much as we can get and of course that is why we have worked with the cow Dr Gassner has developed a very nice operation for putting a cannula into the adrenal sinus of the cow and he has been able to collect 5 to 10 liters of blood from a cow which has been stimulated by ACTH (5)

Astwood Have you had enough of the highly oxygenated compound to test biologically to see whether it is an active adrenocortical substance?

Nelson We have had enough of the material but so far have only tested for androgenic activity biologically

Bloch Is there any evidence for conjugation of compound F?

Nelson Apparently not

Conn In the peripheral blood of man given ACTH B and F have been identified What are their quantitative relationships?

Nelson We have not identified B in the peripheral blood of man as yet just F and that is on the basis of paper chromatography (4)

Sayers Did you say you had looked for the androgenic compound in dog's blood?

Nelson I said we had found a compound having the $\alpha-\beta$ unsaturation which comes off the column before F We have never obtained large enough quantities of it to do biological assays or a more specific characterization

White Isn't it the cow which has the large amount of fecal androgen?

Nelson That is right

White And you found the androgenic material in the adrenal vein blood of the cow?

Nelson Yes I should like to spend a brief time reviewing the methods which have been used in the measurement of adrenal steroids in blood These methods can be divided briefly into the biological and chemical Each of these general groups has its advantages and its disadvantages The biologic methods determine only one specific type of biologic activity but they will measure all the compounds present which have that specific type of activity whereas the chemical techniques are usually dependent upon compounds having a certain group and meas

dose of ACTH the same as those released after a large dose?

Nelson Not as far as we know Most of our isolation work has been done with animals which were treated with fairly large doses of ACTH

Sprague You said that the more polar compound may be related to the amorphous fraction What is your reason for this?

Nelson No definite evidence just the fact that it is a highly oxygenated compound which apparently is more polar than compound F and we have speculated that it might be related to the amorphous fraction

Fremont Smith Would you tell us which animals you are using and something about the technique of blood collection?

Nelson We have chiefly used the dog and the cow following essentially the procedure that Vogt has described A cannula is placed in the lumbo adrenal vein and then the communication from the adrenal gland into the inferior vena cava is tied off so that any blood flowing from the adrenal gland will flow back out through this cannula When ACTH is administered we have been able to demonstrate marked increases in the quantities of hormones being secreted and also to identify corticosterone and 17 hydroxycorticosterone and the androgenic compound from the cow material In the dog we found less polar compounds which had the $\alpha-\beta$ unsaturation or the 240 mμ adsorption We did not obtain large enough quantities to actually identify them

Fremont Smith How much time elapses after the injection of ACTH before there is a noticeable increase in the output?

Nelson We were collecting our samples over ten minute periods and the first sample after the intravenous administration of 10 to 15 mgm ACTH showed a significant rise in the quantity of adrenal compounds in the blood

White In which species was this more polar compound?

Nelson That was in the dog

White Would you care to discuss what you feel is the best technique for separating steroids from blood?

Nelson I would be glad to tell you the techniques we have been using I am not sure they are the best We have used an ether chloroform extraction followed by separation of steroids from most of the lipids via partitioning between 70 per cent ethanol and hexane and then between 30 per cent ethanol and hexane This material was then acetylated

White Do you have any comment regarding what you think you are not getting out?

Nelson The only comment I have is that if there were very small quantities of other compounds we would not have found them because we were working with small quantities as it was of compound F and corticosterone

TABLE VII

Levels of Adrenal Hormones Found in Blood by a Number of Investigators

Investigator	*Method of Assay*	*Source of Blood*	*Collecting Conditions*	Quantity Steroid Found
Vogt	Cold protection test in terms of cortisone	Dog adrenal vein	Abdominal operation	10 20 γ/ml plasma
		Peripheral artery of dog and cat	Abdominal operation	Little if any present
Corcoran and Page	Formaldehyde liberated in terms of DCA	Human peripheral vein	At rest	1 1 4 2 γ/ml plasma
		Dog adrenal vein	Abdominal operation	8 8 48 γ/ml plasma
		Peripheral vein of dog	At rest	1 2 38 γ/ml plasma
Paschkis Cantarow Walking Boyle	Glycogen deposition test expressed as micrograms CMPD A	Dog adrenal vein	*Through London cannula at rest*	14 50 γ/ml plasma
		Dog peripheral artery	At rest	12 1 γ in 1 ml plasma
Porter and Silber	Phenyl hydrazine sulfuric acid color reaction	Peripheral vessels of dog and rat	Before and following cortisone administration	No demonstrable levels
Nelson and Samuels	240 mμ absorption for $\alpha\beta$ unsaturation	Dog adrenal vein	Abdominal operation	10 20 γ/minute 5 10 γ/ml blood
	Porter Silber phenyl hydrazine sulfuric acid color reaction	Human peripheral vessels	At rest	4 10 γ/100 ml blood
		Addison s Disease	At rest or following I V ACTH	<1 γ/100 ml blood
		Cushing s Disease	At rest	15 21 γ/100 ml blood

ure all the compounds having that group but not necessarily those having similar activities

Table VII is an outline of some of the methods which have been used for estimation of adrenal steroids in blood Vogt has used the cold protection test (5) Hemphill and Reiss (6) have employed a method utilizing the reducing properties of the side chains of the cortical steroids Corcoran and Page (7) have used formaldehyde formation on oxidation with periodic acid and Paschkis (8) employed glycogen deposition in adrenalectomized mice We have used the adsorption at 240 $m\mu$ in the ultraviolet spectrum and the Porter and Silber (9) phenylhydrazine sulfuric acid reaction for 17 21 dihydroxy 20 ketones

Vogt using the cold protection in adrenalectomized rats was able to detect these steroids in adrenal venous blood She was handicapped however by this method both because of relatively low sensitivity and because of the fact that only changes of approximately a threefold nature could definitely be ascertained The quantities obtained in adrenal venous blood by Vogt have been estimated as being equivalent to approximately 20 gamma per milliliter of cortisone secreted by one dog s adrenal per minute These values of course were found when the dog was under considerable stress as it had previously been operated on for the insertion of the cannula Vogt was not able to find any consistent level of adrenal steroids in peripheral blood although on one occasion she apparently found some activity in cat blood

Paschkis and his co workers (8) have generally confirmed Vogt s findings with adrenal venous blood by means of the liver glycogen deposition test and have also demonstrated activity in peripheral blood of human patients They have reported levels of adrenal steroids in peripheral blood to be 12 gamma equivalent of compound A acetate per milliliter of plasma However in their paper they report their uninjected mice as having a value of 11 6 gamma equivalent of compound A acetate No dose response curve was given

Corcoran and Page (7) using periodic acid oxidation of partially purified plasma extracts to demonstrate the formaldehyde produced have reported levels of 1 1 to 4 2 gamma of DCA equivalent per milliliter of plasma These values seem rather high in terms of what we have found

Porter and Silber (9) have described a color reaction which is relatively specific for compounds having the dihydroxyacetone type of side chain or in the case of steroids the 17 21 dihydroxy 20 ketones They have applied this color reaction to extracts of plasma from dogs and from rats and have reported that they were not able to measure any level of 17 hydroxycorticosteroids in the blood of these animals They also administered 100 mg of cortisone acetate subcutaneously to their

significant levels of adrenal steroids in peripheral human blood However through the application of a micro modification of the color reaction described by Porter and Silber to similar extracts we have been able to demonstrate levels of 17 hydroxycorticosteroids ranging from 4 to 10 gamma per 100 mm of blood or about 07 to 0 18 gamma per ml of plasma which is much lower than those reported by Corcoran and Page and also by Paschkis The color reaction in our hands is accurate in the measurement of quantities ranging from 25 to 10 gamma of compound F and recoveries of compound F added to blood or plasma in the range of 2 to 6 gamma are about 80 per cent when carried through the entire procedure

I should now like to refer back to Table VII As can be seen Vogt was able to obtain quantities of steroid in plasma of adrenal venous blood which correspond roughly with those which we have found are not far from what Paschkis found and are not remarkably lower than what Corcoran and Page reported However in the measurement of steroids in peripheral blood there is not quite as good agreement Vogt found little if any apparently due to the lack of sensitivity of her method Corcoran and Page on the other hand found 1 1 to 4 2 gamma per ml of plasma and Paschkis and his co workers found levels of about 12 gamma per ml of plasma

We have found much lower levels We have been able to establish that in cases of Addison s disease there is no demonstrable level of 17 hydroxycorticosteroids as measured by our methods in the blood and that in one case of Cushing s disease on two separate occasions we found levels of 15 and 21 gamma per hundred ml of blood levels which are definitely above the normal range This patient was operated on and found to have adrenal hypertrophy She had a typical clinical picture of Cushing s disease

Would anyone care to discuss what I have said thus far?

Harris Do you agree with the results on adrenal vein blood found by Dr Bush in England?

Nelson Dr Bush has also identified compound F in the adrenal venous blood of the dog (11) We have corresponded with him and I understand he too has found corticosterone and 17 hydroxycorticosterone in various species but he has not published that yet and as I am not acquainted with all the details of his work I would rather not attempt to discuss it He has also found increased levels of compound F in one case of Cushing s disease if I am not mistaken

Pincus Dr Zaffaroni published with us on the cortical steroid content of cow blood Since this was cow blood taken at the time of slaughter it certainly did not represent venous blood per se but the content of cortical steroid measured by paper chromatography tends to

dogs and 1 mg of cortisone acetate per 250 gram body weight intra peritoneally to rats and still were unable to demonstrate any level of adrenal steroids in the blood of these animals

Our extraction partitioning and chromatographic procedure is illustrated in Table VIII We extracted whole blood with ether partitioned between ethanol and hexane dried the ethanol *in vacuo* chromatographed on magnesium silicate and took various fractions which *may or may not have contained these steroids* When we first started with these determinations rather than the Porter Silber color reaction we were using the adsorption at 240 millimicra in the ultraviolet to determine the quantities of steroid present By means of this method we were able to demonstrate levels of 10 to 20 gamma of adrenal steroids being secreted per minute per adrenal gland in the dog under the conditions of the operation (10) We were also able to demonstrate a marked rise in these alpha beta unsaturated compounds following the administration of ACTH These values correspond roughly to those of Vogt

Although this method gave adrenal steroid levels as low as 1 gamma per milliliter of plasma or a little less we were not able to demonstrate

TABLE VIII

Method by which Adrenal Steroids are Measured in Blood

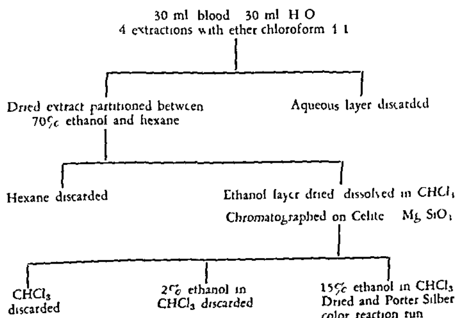


Figure 21 shows that when 15 mg of ACTH is administered intravenously to a human subject there occurs a prompt rise and fall in the compounds in the peripheral blood (On all of these occasions we included simultaneous arterial and venous samples to see if we could demonstrate any significant and consistent difference) Figure 22 demonstrates a similar picture when 25 mg of ACTH were given intramuscularly the rise was a little slower and the level stayed up slightly longer but the picture is quite like the previous one Figure 23 illustrates the effect of ACTH administered intramuscularly subcutaneously and then by intravenous infusion on successive days to a patient with rheumatoid arthritis This graph is somewhat misleading The ACTH was being given every six hours and some of the values shown were taken at the end of the six hour period However it demonstrates that the elevated level was not being maintained by the administration of the ACTH rather than that the ACTH was not having an effect at these points You can see we were giving as much as 120 mg of ACTH per day and obtaining only very slight rises in the levels of adrenal steroids in the blood but when the ACTH was given by intravenous infusion we found very marked rises Approximately four hours after the in

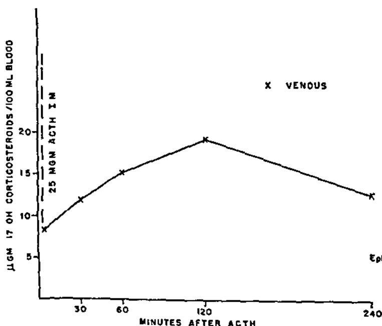


FIGURE 22 Reprinted by permission from Nelson D H *et al* The levels of 17 hydroxycorticosteroids in peripheral blood of human subjects *J Clin Endocrinol* 11 *1021 (1951)*

be rather higher than the figures you have given here

Bauer The cow having been hit on the head and the neck cut during the process of slaughtering!

Hechter I think it is only fair to say there is quite a variable picture in the corticosteroid content of cow blood obtained at slaughter In one instance for example it might be possible to obtain evidence for a variety of ketols in sizable amount including 11 desoxycorticosterone Then in another blood sample collected and extracted under similar conditions nothing would be detectable In all cases however, where ketol steroid is present the major components uniformly prove to be corticosterone and 17 hydroxycorticosterone

Sayers What are the principles of Bush s technique?

Nelson His publication describes a different type of paper chromatography than that which Dr Zaffaroni has used He employed aluminum oxide impregnated paper for his chromatography but I understand that he now uses the method described by Dr A Zaffaroni and Dr R B Burton

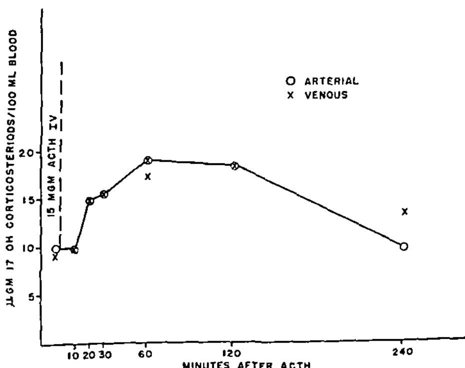


FIGURE 21 Reprinted by permission from Nelson D H *et al* The levels of 17 hydroxycorticosteroids in peripheral blood of human subjects *J Clin Endocrinol* 11 *1021 (1951)*

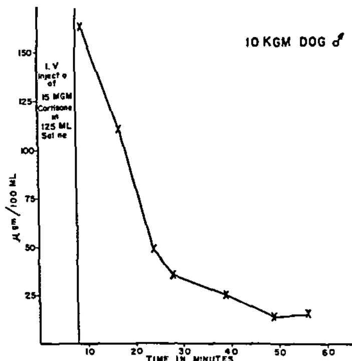


FIGURE 24 Reprinted by permission from Nelson D H Samuels L T and Reich H The cortical steroid in mammalian blood after ACTH stimulation *Proc Second Clinical ACTH Conference Vol 1* Mote J R Editor Philadelphia The Blakiston Co 1951 (p 49)

Figure 25 is a similar picture in a 74 kg man to whom we gave 200 mg of free cortisone intravenously Again there was a marked drop in levels of the compound in the blood stream

When we found that the levels of cortisone in the blood stream dropped so rapidly following injection of large quantities of the steroid Mr Harding and I tried to determine just where this cortisone was going As you know when fairly large doses of cortisone are administered intra muscularly or orally only small percentages of the administered cortisone can be isolated from the urine We thought it would be interesting to see what happened to the cortisone as it passed through the kidney Therefore we made simultaneous determinations on blood obtained from an artery usually from the carotid artery and from blood as it came from the adrenal vein to study arteriovenous differences across the kidney and demonstrate any removal of the compound by the kidney *Figure 26 shows that the arterial and renal venous levels stay very close* together as the steroid level in the blood changes Apparently in the

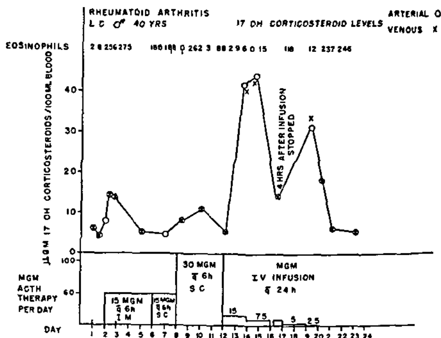


FIGURE 23 Reprinted by permission from Nelson D H *et al* The levels of 17 hydroxycorticosteroids in peripheral blood of human subjects *J Clin Endocrinol* 11, *1021 (1951)*

fusion was stopped the level had fallen almost, but not quite to normal Intravenous infusion of only 5 mg given over a period of twenty four hours again raised the level considerably above normal and even 2 5 mg of ACTH given in the infusion over twenty four hour period main tained the level significantly above that found in the normal individual The last two values were taken after the therapy was stopped and were at the pretreatment level

Hechter I see eosinophils riding along

Nelson There were some eosinophil measurements during the pro cedure but as you can see they did not always follow the therapy close ly However on no occasion was more than one determination made during the day so too good a correlation would not be expected I shall speak more about that later

If 15 mg of free cortisone in 125 ml saline are administered intra venously to a 10 kg dog Figure 24 illustrates what happens to the blood level of these compounds We were able to obtain markedly elevated levels of the compound in the blood of the dog As you can see however the drop was sharp and at the end of an hour there was little of the injected cortisone still circulating in the blood stream

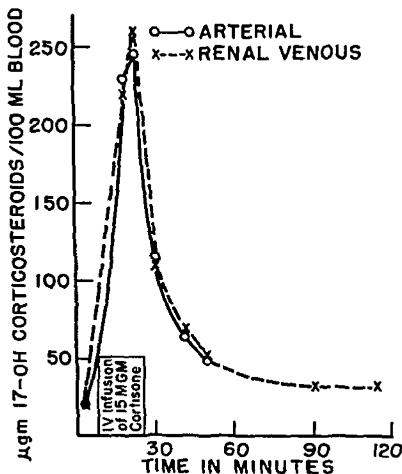


FIGURE 26 Differences between arterial and renal venous blood levels of 17 hydroxycorticosteroids following I V administration of 15 mg cortisone to a 9 kg dog

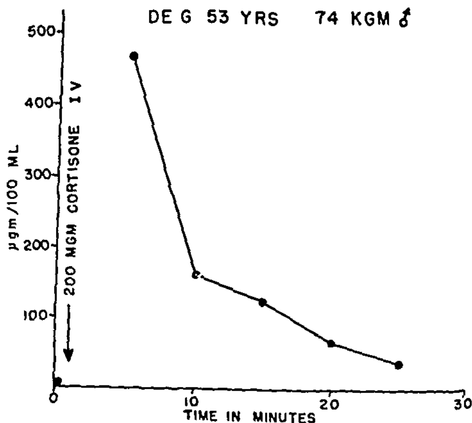


FIGURE 25 Reprinted by permission from Nelson D H Samuels L T and Reich H The cortical steroid in mammalian blood after ACTH stimulation *Proc Second Clinical ACTH Conference Vol I* Mote J R Editor Philadelphia The Blakiston Co 1951 (p 49)

Thorn Do you have determinations on portal vein blood?

Nelson No we haven t Those are now under way Dr Thorn

Figure 28 illustrates the effect of the administration of 200 mg of F acetate orally to a normal subject There is a marked rise in the levels of these compounds in the blood stream the peak apparently being ob tained in this case at one hour It is rather surprising to find that when similar dosages are given intramuscularly elevations of a similar degree do not occur the level fails to show much change On two occasions we have had rises from an initial level of 5γ per cent to 9γ per cent staying within what we consider a normal range but we have never

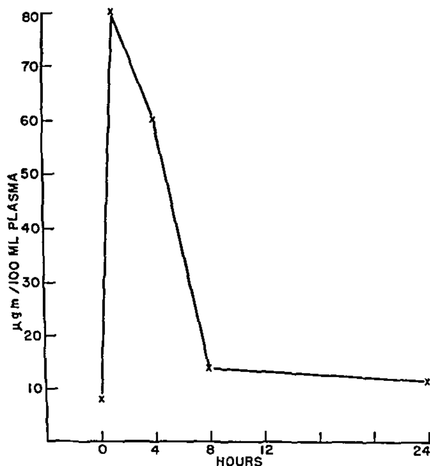


FIGURE 28 Graph showing effect on blood levels of 17 hydroxycorti costeroids of 200 mg Compound F given orally to a normal male subject

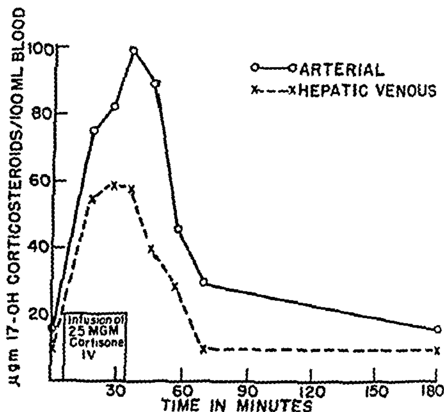


FIGURE 27 Differences between arterial and hepatic venous blood levels of 17 hydroxycorticosteroids following IV administration of 25 mg cortisone to a 13 7 kg dog

dog at least the kidney has very little to do with the rapid removal of these compounds from the blood stream

Figure 27 illustrates the findings of a similar experiment except that in this case the venous blood was obtained from the hepatic vein The initial level as we obtained it here was somewhat higher than normal in contradistinction to the other animals which we have studied and there was a significant arteriovenous difference before the cortisone was injected Then as you can see at the peak there was a marked difference between arterial and venous samples and this difference was maintained as the cortisone was being removed from the blood stream

We do not really know where the cortisone is going We only know that as it passes through the portal system it is altered removed or conjugated in some way so that we are no longer able to measure the same level in the hepatic venous blood as in the arterial blood at a similar time

Therefore possibly in the peripheral blood you are measuring something which has a 21 ketol side chain but it may not necessarily be F

Nelson You mean in the case of oral administration?

White Yes You call your determinations F They are actually 17 hydroxycorticosteroids

Nelson That is what we have preferred to call them

White I am trying to suggest that possibly the active substance and perhaps the eosinophil counts fit in with the idea that the active material is not F but a metabolic transformation product not a new suggestion I am sure

Thorn I think it is an interesting one Dr White but I would feel that the eosinophil titration system would not be a good one to prove this point In other words we interpret a delay in the appearance of the eosinopenia in comparison to the elevated levels of 11-oxysteroids as indicative of a time lag between the decrease in the reserve of circulating eosinophils These cells as we all know are being produced at a regular rate and one would expect a time lag between the attainment of maximum blood levels of hormone and the maximum fall in eosinophils If one were thinking in terms of a fixed number of eosinophils in the circulating blood stream without the addition of new cells then the delayed reaction could be interpreted as suggesting the necessary conversion of cortisone or compound F to an intermediary substance but I do not think it is possible to make this interpretation in a system in which there is a constant addition of cells to the circulating blood

White It occurs to me that a very simple test would be to inject the metabolic transformation products into an adrenalectomized preparation to see what happens to the eosinophils

Thorn It would be better to give the substance by mouth since we have shown very rapid and effective absorption of the 17 oxysteroids by this means In substantiation of Dr Nelson s observations we can state that the four hour peak level in the blood is well established in the orally administered hormone experiments that we have carried out I think Dr Nelson s blood assays give an explanation for the difference in the marked eosinopenia which we have observed immediately following the administration of oral cortisone in contrast to the very poor eosinopenic response with intramuscularly injected cortisone While we are discussing this I would like to reiterate the point which I made in regard to Dr White s question concerning lymphocytes I feel it is very likely that the lympholytic action of the 17 hydroxy compounds is as great or greater than the eosinopenic action but the reserve of lymphocytes in the lymph nodes and the production of lymphocytes is so large that one is not able to produce a striking change in blood level because of the reservoir of these cells as compared to the changes in blood

found any marked rise when the material is given intramuscularly We have followed the eosinophils while doing this, and they agree very well When determined after cortisone is given orally as Dr Thorn has also observed there is a marked drop in the eosinophils whereas when it is given intramuscularly there is a slight and sometimes an equivocal drop in the eosinophils

Another interesting finding is that the peak level of steroid in blood occurs in the neighborhood of an hour to an hour and a half but the fall in the eosinophils when we were measuring them at one two and four hours did not occur until the fourth hour In three cases now, which we have followed this effect has been delayed in relation to the rise in blood levels of corticosteroids The relation between blood levels of 17 OH corticosteroids and eosinophils following 200 mg of F acetate given orally to a normal individual is illustrated in Table IX The eosinophil drop was not found until the steroid level had passed its peak and started to fall This is not an isolated case but appears to be a fairly consistent finding

Kendall Is the weight expressed in gamma?

Nelson This is gamma per hundred milliliters of blood

White This is in a human subject given the material by mouth?

Nelson That is right

White You haven t done similar experiments with F and F acetate injected?

Nelson We have with the F acetate injected but not with the free F

White There appears to be evolving the suggestion that compound F is transformed in the liver into something which may no longer be F

TABLE IX

	17 OH corticosteroids γ per/100 ml blood	Eosinophils per cubic mm
Control	4 0	269
200 mg F acetate given orally		
½ hour	24 0	269
1 hour	[illegible] 0	288
2 hours		234
4 hours	35 0	13
8 hours		19
24 hours	3 0	225

lites of greater activity than cortisone may even be formed While this is speculation I think it is in line with the evidence at this time

I believe Dr Nelson that you said you had injected 15 mg cortisone into a 10 kg dog and measured the cortisone levels in the blood at various intervals I recall from Figure 24 that your highest value for blood cortisone was obtained about ten minutes after injection and amounted to 160 micrograms per 100 ml Ten minutes seems a fair time for blood mixing If you estimate that a tenth of the body weight represents blood volume one may calculate about 1 6 mg of cortisone circulating in the vascular bed ten minutes after injection This represents about 10 per cent of the dose administered What happened to the other 90 per cent? Your A V differences on muscle or kidney reveal no significant utilization of cortisone The only A V differences that you find to be important are those in liver All of this again reinforces the notion that cortisone is rapidly metabolized presumably in greatest part by the liver your data suggests that 90 per cent of it is removed from the circulation within ten minutes or so

Nelson That of course, was our point and the reason for undertaking this study with the A V differences was to find out just what was happening to the cortisone We have been puzzled ourselves by this rapid removal We have never been able to account for all of it immediately at the end of infusion Of course it has taken about ten minutes to infuse that quantity into the dog

Ingle I can see that I shall have to renew my studies on the biological effects of these steroids in the liverless rat Adrenal cortex extracts do have easily demonstrable effects upon carbohydrate tolerance and upon the level of plasma amino acids in the eviscerate rat but we were surprised and disappointed to find little or no effect of cortisone upon the glucose tolerance of the eviscerate rat Perhaps there is a reason for it

Sayers Was cortisone given to the liverless animal intravenously?

Ingle Yes

Sayers The adrenal cortical extract was effective?

Ingle Yes

Hechter Dr Ingle are there any other deficiencies exhibited by this liverless animal with respect to cortisone?

Ingle Cortisone does cause some rise in plasma amino acids in the eviscerate rat but the extent of the response was less than we had expected The adrenalectomized eviscerate rat does show a fall in plasma amino acids which can be corrected by the administration of cortisone We have no side by side comparisons of extract and cortisone

Nelson To return to the account of the work we have done I should like to point out that we are very much aware that the technique

level of eosinophils However, I believe that examination of the lymph nodes and thymus might show marked involution during administration of these hormones

White There is another suggestion that arises which I am sure will be promptly shot full of holes I wonder if the observations possibly afford an explanation for some of the differences which may exist in the comparative parenteral effectiveness of F versus F acetate that Dr Conn has described as contrasted with the oral effectiveness of the two compounds It is striking in isolated organ perfusion experiments that the capacity of the organ to transform the steroid is much greater in the case of a free alcohol than it is in the case of the acetate and orally administered or parenterally administered materials might be expected to show greater differences when one compares the free alcohol with the acetate than orally administered materials which have a possibility of prior hydrolysis in the gastrointestinal tract

Thorn I should like to take the first shot The only way we have been able to demonstrate any difference between the combined and free forms is our study of the eosinophil response to orally administered hormone The free form is more effective F is more active than E and the free F is more active than the F acetate

Hechter We have been working for more than a year on the hypothesis that some intermediary metabolite arising from cortisone might well be an active biological agent Along this line we have perfused a variety of corticosteroids but principally desoxycorticosterone and cortisone through rat and rabbit liver We find a very rapid disappearance of the added steroids In the case of desoxycorticosterone where rather large amounts have been perfused through liver only trace amounts remain Cortisone is transformed more rapidly if anything than is desoxycorticosterone To date we have been concerned primarily in attempting to characterize the transformation products arising from desoxycorticosterone and cortisone We find that there is a multiplicity of products perhaps more than twenty with desoxycorticosterone

These perfusion findings in association with other studies which have been mentioned may be of significance On the one hand from clinical studies the picture seems to be clear that compound F and cortisone are about as active or perhaps slightly less active orally as parenterally This observation would suggest that the biological activity associated with these steroids is relatively resistant to hepatic inactivation On the other hand from the *in vitro* perfusion evidence it would appear that cortisone is rather rapidly transformed This strongly suggests that a metabolite arising from the action of liver enzymes upon cortisone may be active biologically If there is a multiplicity of products formed *in situ*, say ten or so all of which do not retain biological activity metabo

stance we come out with figures that vary from approximately 3 to 7 mg per liter or if you want to use 100 ml that would be 300 to 700 micrograms per 100 ml This is you see a factor of approximately a hundred times the figure Dr Nelson has talked about We then proceed to fractionate this material crudely by putting it on silica gel columns and taking off initially an early fraction which comes off with benzene and which we know from our previous studies contains little or no steroid

Kendall Is this an acetate?

Pincus This is the free form and we proceed to use various benzene ethyl acetate mixtures finally ethyl acetate and then we strip the column with methanol The middle fractions the ones which combine the benzene ethyl acetate mixture and ethyl acetate contain now from about 200 to 500 micrograms per 100 ml still a lot more than Dr Nelson finds This is the formaldehydogenic reaction This reaction you recall involves the oxidation of the side chains to produce formaldehyde and will occur either with ketols or with glycols therefore there is a possibility of four major types being present only one of which was measured Dr Nelson measured the 17 hydroxyketone the others possibly present are 17 hydroxyglycol and the 17 desoxy ketol and glycol

If we then take these middle fractions which contain the steroids and put them on paper using the Zaffaroni method what we find is that in the neighborhood of—and here the figures will have to be very rough because we are right in the midst of the work—5 per cent of that material is compound F by this criteria Thus we come down from 200 to 500 micrograms of total elutes to 10 to 25 micrograms of F in human peripheral blood That figure of 5 per cent might be as little as 2 per cent it can t be much less and it can t be much more than 5 per cent So now we are almost in agreement We find a little bit more I suspect in part due to the fact that our method of extraction possibly removes somewhat more material than yours although it is hard to say Your recovery data are certainly just as good as ours so the discrepancy cannot lie there It may be that the human blood is from stressed individuals because this is blood taken at a blood bank and when the people came to the blood bank they may have been emotionally stressed

The interesting question is the identity of the other 95 per cent of material which registers in this formaldehydogenic reaction We do not know that at the present We find material which moves more slowly than F on paper one component which we suspect is dihydro E

Kendall 4 5 dihydro E?

Pincus The three ketone This is about as far as we have gone We have indications of the presence of a couple of other substances As

we are using now measures only one type of compound only those compounds which have the 17 hydroxyl group but from our addition experiments to blood and plasma we feel that we are measuring most of the F type of compound which is circulating in the free form in peripheral blood It has been rather difficult for us to see how there could be such high levels reported by other workers Using ultraviolet adsorption we certainly would have found levels of 4 γ per milliliter of plasma Since we may not have found levels in the neighborhood of one half of a gamma per milliliter of plasma with that technique there may be considerably more of these adrenal steroids circulating in the blood than we are measuring Our own evidence from the isolation from adrenal venous blood has been that F is the chief compound

Long Have you made measurements of this kind after for example putting the dog in the cold for three or four hours?

Nelson We have not done those experiments yet although we plan to

Long I think you mentioned at the Laurentian Conference that with electroshock treatment you observed an immediate rise

Nelson Yes that is correct In fact the very first studies we did with this technique were on measurement of blood levels before and after electroshock was administered to patients on the psychiatric ward Within a period of ten or fifteen minutes there is a rise in the level of these steroids in the blood

Long Have you and Dr Sayers discussed this question? I am trying to see what the differences of opinion are on this point since Dr Sayers has suggested that the fall in the blood level of adrenal cortical hormone acts as a regulator of ACTH secretion You have perhaps not done experiments which Dr Sayers would consider critical in this regard

Nelson Certainly if epinephrine is an important factor that can explain the rise because these patients are under considerable stress and their adrenals are discharging epinephrine when the shock is given

Long That is also associated with muscular contraction is it not?

Nelson Yes

Pincus I should like to say a few words about the quantitative discrepancies which you recognize and which as a matter of fact are pertinent to much of what has gone before We have been doing some measurements of cortical steroid in human peripheral blood and also in human blood perfused through the adrenal gland The latter I don t want to talk about now One phenomenon has shown up which may reconcile many of the discrepancies If we extract the blood by the method which we have used with the perfused blood namely by extraction with charcoal and then elute the charcoal with the proper solvents and so on then measure the total formaldehydogenic sub

tions obtained therefrom Much to our dismay we found that there were large amounts of formaldehydogenic material in the nonperfused blood amounting to several milligrams per 100 ml One fraction of this material which seemed to be phospholipid was assayed for glycogenic activity because of its rich formaldohydogenic activity but it proved completely inactive Thus we became convinced very early in the game that we would have to remove this material in normal blood which gave rise to formaldehyde following periodate oxidation

Chloroform extraction of blood was investigated next and it was found that added corticosteroids could be recovered in good yield However direct chloroform treatment extracted less of this noncorticoid formaldehydogenic material The chloroform residue was fractionated between pentane and 70 per cent ethanol the aqueous alcohol brought to dryness and the corticosteroids dissolved in acetone in the cold (The white precipitate which came out was discarded) With this technique our values for normal nonperfused blood became lower approaching about 50 to 150 micrograms per 100 ml

We went on to dialysis of blood and found that we could recover just about as much corticosteroid as with the other methods However little or none of this noncorticoid formaldehydogenic material passed through the membrane The values of formaldehydogenic content in normal blood were in the range of 50 to 100 micrograms per 100 ml blood

The point I am raising is that perhaps the difference in these various procedures which you described Dr Nelson does not lie in the fact that some people use formaldehydogenic steroid determinations while you use the Porter Silber reaction but has to do with differences employed to extract and purify the corticosteroid concentrate used for chemical estimation

The nature of this material present in normal blood which gives rise to formaldehyde following periodate oxidation initially thought to be phospholipid proved on silica gel chromatography to be more polar than any of the known cortical steroids One fraction of this material weighing 50 mg had a formaldehydogenic equivalent of 89 mg of desoxycorticosterone From this we concluded that we were probably dealing with a small highly polar molecule with a ketol or glycol grouping We do not know what it is but it is entirely possible that some of the workers who have reported on corticosteroid values in blood have been measuring this material in part

Nelson How much biologic activity were you able to extract per hundred milliliters of blood?

Hechter No studies of this type were done

Li Dr Nelson have you been using graded dosages of ACTH to

yet we have no good methods, unfortunately for identifying glycols which is what we would like to have

Kendall Have you used silver?

Pincus We have not used that We have used tetrazolium reagents

Hechter Glycols do not react with tetrazolium reagents

Pincus As far as we can see it looks as though a large amount of material in the peripheral blood is glycol Whether it is steroid is still problematical

Thorn Did you carry out a biological assay?

Pincus Not yet no I might say this is Dr Thorn s blood so he is obviously interested

Long His personal blood?

Pincus His contribution let s put it that way

Thorn Somebody else s blood but my sweat

Pincus And our tears We did find a small amount of material but again 1 or 2 per cent at most which gives the iodine reaction As Dr Thorn knows we are mystified by what this strange material must be because originally, our figures agreed fairly well with those of Corcoran and Page (7) and we thought we were really getting steroid Now we are not sure that we are I feel in a way much more confident with the figures that Dr Nelson gives from the point of view of what is identifiable and probably good steroidal material than I feel with these formaldehydogenic determinations On the other hand the formaldehydogenic determinations do suggest that there may be a very interesting nonketol

Nelson Would your method of separation remove all the phospholipids that are present?

Pincus Do you want to talk about that Dr Hechter?

Hechter The work which Dr Pincus has been referring to has been carried out primarily by Dr R Dorfman and his group at Worcester Foundation I do not know the details of all their findings I should like to make some remarks about our early experiences in attempting to extract cortical steroids from blood When we started no arbitrary assumptions were made about the nature of the corticosteroids released from adrenal glands into the circulation We were prepared to consider the possibility that cortical hormones might be secreted not as free steroids but conjugated with ascorbic acid for example With this in mind we set up a simple type of experiment Two portions of blood were taken One was perfused through an adrenal gland with ACTH and then various fractions from both blood samples were analyzed for formaldehydogenic steroid content using desoxycorticosterone as standard Originally we used a technique which involved alcohol ether or acetone extraction of blood with subsequent workup of the lipid frac

F and E and perhaps corticosterone may be taken off in your early fraction?

Nelson All of our work has been dircted towards the identification of what we think of as active steroids Our recovery experiments have been done with F corticosterone and desoxycorticosterone so we really do not know We did not measure compounds such as you mention the 20 hydroxyl rather than 20 ketone or ones not having the 3 ketone If present in very small quantities we would not have been aware of them

Thorn The 200 mg of F acetate was given to an individual with intact adrenals I assume

Nelson Yes it was

Thorn The low values observed following the intramuscular injection could be accounted for by compensation on the part of the adrenal itself For example the Addisonian patient might show a 5 microgram rise following the standard dose of compound F while an individual with an intact adrenal might compensate for the administration of the synthetic hormone by decreasing his endogenous production of the hormone

Nelson The changes are so slight and we have observed them on such few occasions that I hesitate to discuss them Actually with the intramuscular injection we found a zero level at the one hour period

Thorn I think that is a very important point

Nelson I should point out something else the difference was not that we could measure the free compound and could not measure the acetate That obviously was the first thing that occurred to us the acetate of course comes off the chromatograph at a different point We went through the process of adding F acetate to blood and found that we could recover it But using the same procedure we were not able to recover the acetate in the case where we were giving it intra muscularly

Sayers How does free cortisone compare with the acetate?

Nelson It is very comparable If anything we have felt the effect on the eosinophils was somewhat greater by giving F rather than cortisone

Sayers You mean as far as the blood steroid level is concerned?

Nelson As far as the steroid and also the eosinophil level

get different levels of blood steroids? Or were you using only a 15 mg dose?

Nelson As yet, we have not tried to grade the response to quantity of ACTH administered

Li It would be interesting to correlate the level of the blood steroids with the ascorbic acid depleting activity of ACTH

Nelson In order to get around the problem of adsorption we gave 200 mg of F acetate intramuscularly to the patient shown in Figure 28 the same dose as was previously given orally We gave 50 mg in four different sites and the results are illustrated in Table X There was no rise in the steroid level When it was given orally there was a marked rise in the steroid level, and there was a fall a delayed fall in the eosinophils Given intramuscularly there was no fall in the eosinophils and there was no rise in the steroid level in the blood

Ralli Is there ever anything that interferes with the intestinal absorption of cortisone or of any of these compounds?

Nelson Not in our experience but our experience is limited of course

Astwood In the experiment where you gave the steroids intravenously in what form were they? Were they in solution?

Nelson In the dog experiment we gave the 15 mg free cortisone in saline solution We find we can get a little over a quarter of a milligram of cortisone into a milliliter of saline if it is shaken in the incubator overnight In the case of the human experiment it was about 5 per cent ethanol solution

Pincus Is there any evidence that your column steroids other than

TABLE X

	17 OH corticosteroids γ per 100 ml blood	Eosinophils per cubic mm
Control	3 0	294
200 mg F acetate given orally		
1 hour	0 0	356
2 hours		400
4 hours	4 4	400
8 hours	4 0	250
24 hours	0 5	331

THE BIOGENESIS OF ADRENAL CORTICAL STEROIDS

OSCAR M HECHTER
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LAST YEAR AT THE Laurentian Hormone Conference we presented data concerning the nature of the adrenal secretory product and the metabolic pathways involved in its elaboration (1) Today I should like to present some of our more recent findings and outline questions which appear to arise from our work

The we that I speak about included initially a group at the Worcester Foundation consisting of Drs R P Jacobson H Levy R Jeanloz C W Marshall V Schencker G Pincus and myself Later we were able to enlist the collaboration of Dr A Zaffaroni and it was largely as a result of his collaboration that rapid progress was made in several phases of the work More recently Drs I Alden Macchi and M Solomon both at the Worcester Foundation have been associated in the work However I should state that I must bear full responsibility for the deductions and more particularly for the unsupported speculations which will arise during this discussion I should also like to acknowledge that my view as to a mechanism of ACTH action at a molecular level derives in great part from discussions with Dr Albert Szent Gyorgyi and represents a variant of Szent Gyorgyi s basic concept (still in the process of development) dealing with fundamental mechanisms of hormone action

May I review first some of our published data Table XI illustrates the scope of the perfusion technique in studying certain questions bearing on steroidogenesis in an isolated *in vitro* system Homologous citrated blood of known corticosteroid content is perfused at a known pressure through an isolated beef adrenal gland for certain time intervals By estimating the corticosteroid content of the adrenal effluent samples (as formaldehydogenic steroid) and by knowing the blood flow it is possible to calculate steroid output in terms of μg per minute before and after certain treatments In Table XI the effect of adding ACTH to this system is shown Without ACTH there is initially a small output of steroid which tends with time to decline toward zero After the administration of ACTH a sizable increase in the output of formaldehydogenic steroid is observed

REFERENCES

1 NELSON D H REICH H and SAMUELS L T Isolation of steroid hormone from adrenal vein blood of dogs *Science* 111, *578 (1950)*

2 REICH H NELSON D H and ZAFFARONI A Isolation of 17 hydroxycorticosterone from blood obtained from adrenal veins of dogs *J Biol Chem* 187, *411 (1950)*

3 GASSNER F X *et al* Isolation of androgenic compound from adrenal venous blood of cows *Proc Soc Exper Biol & Med* *77, 829 (1951)*

4 NELSON D H *et al* Levels of 17 hydroxycorticosteroids in peripheral blood of human subjects *J Clin Endocrinol* 11, *1021 (1951)*

5 VOGT M Output of cortical hormone by mammalian supra renal *J Physiol* 102 *341 (1943)*

6 HEMPHILL R E and REISS M Regulation of endogenous cortin production *Endocrinology* 41, *17 (1947)*

7 CORCORAN A C and PAGE I H Methods for chemical de termination of corticosteroids in urine and plasma *J Lab & Clin Med* 33, *1326 (1948)*

8 PASCHKIS K E *et al* Adrenal cortical hormone levels in adrenal vein—and peripheral blood *Endocrinology* 47 *338 (1950)*

9 PORTER C C and SILBER R H A quantitative color reaction for cortisone and related 17 21 dihydroxy 20 ketosteroids *J Biol Chem* 185, *201 (1950)*

10 NELSON D H SAMUELS L T and REICH H The cortical steroid in mammalian blood after ACTH stimulation *Proceedings of the Second Clinical ACTH Conference* Mote J R Editor Philadelphia The Blakiston Co 1951 (p 49)

11 BUSH I E Hormones in the adrenal venous effluent *J Physiol* 112 *10P (1951)*

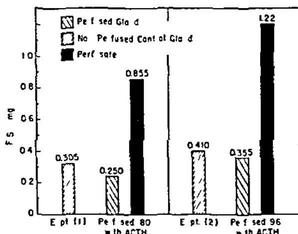


FIGURE 29 The results of two typical individual experiments demonstrating that isolated adrenal glands perfused with ACTH synthesize corticosteroid estimated as FS under *in vitro* conditions Reprinted by permission from Hechter O *et al* Nature and biogenesis of adrenal secretory product *Recent Progr Hormone Research* 6 215 (1951)

Table XII illustrates the concentration of various steroid ketols present in an ACTH perfusate relative to an adrenal perfusate from the same gland using the same blood without added ACTH For comparison the steroid content of a sample of this blood prior to perfusion through the adrenal is also shown There are fifteen steroid ketols present in these ACTH perfusates Of these two steroids are present in greatest concentration 17 hydroxycorticosterone and corticosterone The thirteen other steroid ketols present in the secretory product elaborated by these isolated glands consist of all of the known cortical hormones (except 17 hydroxy 11 desoxycorticosterone) and ten unknowns of varying polarity five of which are more polar than 17 hydroxycorticosterone Despite the presence of a multiplicity of steroid ketols in the adrenal secretory product of perfused bovine glands the two principal components are 17 hydroxycorticosterone and corticosterone These same two steroids also appear to be the major steroids present in the adrenal venous blood of animals of a variety of species* (3 4) On this basic point therefore the *in vitro* perfusion data are in full agreement with *in situ* studies

Measurable amounts of corticosteroid are present in some bovine

* ZAFFARONI A Unpublished observation

TABLE XI

Output of Corticosteroid in a Single Perfused Cow Adrenal

Sample	Period of perfusion (min)	Average blood flow (ml/min)	Average arterial pressure (mm Hg)	Corticosteroid	
				Concentration (µg/100 ml)	Output (µg/min)
Initial	0	—	—	52	—
2	20	5 0	40	239	9 4
3	37	5 1	40	160	4 3
4	41	4 9	40	66	0 3
Then inject 2 0 mg % ACTH					
5	32	5 2	40	1352	47 2
6	57	3 5	40	1876	33 0
7	90	2 2	40	2606	29 0
8	60	3 3	90	2492	41 8

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This formaldehydogenic steroid which consistently appears in adrenal perfusates following ACTH treatment might arise from corticosteroid fixed in the gland in one form or another which is merely released by ACTH on the other hand it might represent newly synthesized steroid *which is released as it is produced* Figure 29 illustrates two experiments which demonstrate that the responses observed result from the biosynthesis of corticosteroid In these experiments the total amount of formaldehydogenic steroid present in a gland perfused with ACTH and its contralateral nonperfused control was *measured relative to the amount of corticosteroid released into the* perfusate In each case the difference in glandular content of formaldehydogenic steroid is slightly lower for glands perfused eighty to ninety six minutes respectively than for the control gland (which was stored in ice immediately after the death of the animal) The corticosteroid *released into the venous effluent greatly exceeds this small difference It thus appears that the corticosteroid synthesized by these* isolated glands is released into the circulation and that little or none of it is being stored in the tissue This finding will take on significance later therefore I am emphasizing it now

What is the nature of the steroidal substances released from these isolated bovine adrenals that we have been measuring as formaldehydogenic steroid? To answer this question adrenal perfusates were extracted either with charcoal or by dialysis and then fractionated by paper chromatography The steroid ketols present were characterized by methods described by Zaffaroni and his co workers (2)

a 20 gm beef gland as determined by Axelrod and Zaffaroni* is shown From the values in Table XII it can be calculated that each component of the ACTH perfusate (with the sole exception of desoxycortico sterone) has been synthesized by this isolated gland In other experi ments involving recycling of the ACTH perfusate clear evidence was obtained that desoxycorticosterone can also be synthesized by isolated bovine glands (5)

The decrease in the concentration of adrenal ascorbic acid and cho lesterol which follows ACTH administration is now well established (6) We thought it would be interesting to determine whether isolated perfused adrenal glands exhibit similar changes in adrenal cholesterol and ascorbic acid in association with the outpouring of corticosteroids produced by ACTH These experiments done in association with Drs F Elmadjian and R Scola were performed in the following manner Blood containing increasing amounts of ACTH was perfused through a single gland at a constant blood flow Each sample corresponding to a particular concentration was collected and analyzed separately for cor ticosteroid content by our dialysis method At various periods during the experiment biopsies of adrenal tissue were removed and cholesterol and ascorbic acid content measured The biopsies were taken from the outermost zones of the cortex care being taken not to include the con nective tissue capsule

In control experiments it was demonstrated that there were no sig nificant differences in the content of either cholesterol or ascorbic acid in the different zones of the cortex of these isolated bovine glands Table XIII illustrates one of these experiments where the release of corticosteroid induced by ACTH was directly correlated with changes in the tissue concentration of ascorbic acid and cholesterol The ma terial used for stimulation in this particular experiment was a sample of growth hormone furnished us by Dr Li which we believe was con taminated by ACTH The problem of contamination of this prepara tion is another question of which I should like to speak later Ignoring this point for the moment and considering the material only as an ACTH preparation (for similar results were obtained with purified ACTH preparations) it is evident that at a concentration of 100 μg per liter the preparation produces a significant rise in the output of corticosteroid Maximal release of corticosteroid was obtained with 10 000 μg per liter During the interval in which corticosteroid is re leased as a consequence of biosynthesis there was no important change in either the adrenal ascorbic acid or cholesterol This finding may in some way be related to the fact that the absolute values of these tissue

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* Unpublished observations

TABLE XII

The α Ketols Present in Adrenal Perfusates

α Ketols	Micrograms per 2 liter samples of blood: Not perfused through adrenal	Adrenal Perfusates: No ACTH	Adrenal Perfusates: ACTH	Micrograms per 20 g beef gland extracts
Unknowns I V	220	80	700	70
17 hydroxycorticosterone	360	145	1100	40
Cortisone	—	25	200	20
Unknown VI[1]	—	40		
Unknowns VII IX	110	45	250	25
Corticosterone	*400*	*230*	*1100*	*70*
Unknown X	—	60	300	35
Dehydrocorticosterone[1]	—		250	
Desoxycorticosterone	120	35	140	—[3]

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[1]Cortisone and unknown VI move to approximately the same position on paper a similar situation obtains for unknown X and dehydrocorticosterone When acetate or propionate esters are formed the known steroids are widely separated from their respective unknowns In some cases the mixtures were not resolved and data were obtained for the complex of cortisone plus unknown VI or dehydrocorticosterone plus unknown X

[2]Not detectable

[3]Not determined

blood samples Table XII shows the results with one such sample obtained at slaughter The bulk of corticosteroid present is 17 hydroxy corticosterone and corticosterone (both present at a level of about 200 μg per liter) in this blood sample When this blood sample was per fused through a gland without added ACTH no net increase in the corticosteroid content over and above that initially present in the blood was observed While we have observed differences between various glands by and large under the conditions of the experiments bovine glands perfused in the absence of ACTH do not release sizable amounts of corticosteroids

However when ACTH is added there is a striking increase in the concentration of each component of the corticosteroid mixture In the last column of Table XII the amount of steroid ketol extractable from

we have found it possible (and even desirable from a practical point of view) to allow hours to elapse between the death of the animal and the initiation of the perfusion instead of attempting to transfer the organ as rapidly as possible

Loewi Do you leave the glands at room temperature?

Hechter Glands are kept at 2° C in a saline citrate solution Recognizing that such adrenal preparations are not physiological of what possible use are these preparations? From our point of view the perfused gland represents a useful biochemical tool Certain *in vitro* reactions involving corticosteroid biosynthesis occur in this isolated system and these reactions may be studied under relatively defined conditions We are interested in reaction rates and the factors which influence these From this point of view perfusion is a method for studying cellular metabolism akin to tissue slices or homogenate methods albeit at a higher level of organization

With all such systems profound limitations exist with respect to the physiologic significance of *in vitro* results Perfusion should be regarded as no more physiologic (although it may be) than a tissue slice or a homogenate experiment The results of a positive *in vitro* metabolic experiment may have physiologic significance although this is not necessarily the case This must be tested experimentally in the living animal Having said this it is immediately apparent that one should not attempt to extrapolate results obtained in perfused glands back to the living animal in a quantitative sense We believe however that some of our qualitative findings may well prove to hold *in situ* Indeed we are gratified to see that much of our perfusion data are in full conformity with *in situ* studies insofar as comparison is possible

Vollmer Do you have any control values on the cow adrenal as to ascorbic acid and cholesterol? It doesn t seem quite fair to make the comparison with the rat in which these are very high

Hechter The only cow adrenals that we have ever had an opportunity to work with represent slaughterhouse material Perhaps Dr Gassner or someone else who has had the opportunity of working with bovines as experimental animals might be in a position to answer your question I recognize full well that my comparison between the rat and the cow may not be fair I feel however that there must be more cholesterol and ascorbic acid in the cow adrenal than these values would indicate

Pincus We have done this analysis on a number of glands and in some glands the values are higher For example I recall one or two where the ascorbic acid was over 100 mg per cent and where the cholesterol was close to 1 per cent

Hechter 0 25 per cent was the highest value for cholesterol

TABLE XIII

Changes in Adrenal Cholesterol and Ascorbic Acid Associated with Steroidogenesis in an Isolated Beef Adrenal

Sample	STH plus ACTH*	Time after initiation of experiment	Corticoid output in Perfusate	Adrenal Ascorbic Acid	Adrenal Cholesterol
	µg per l	Minutes	mg FS per hr	mg %	%
1	0	0	1 0	76	0 14
2	01	50	0 55	63	0 08
3	10	130	0 92	57	0 12
4	100	155	3 99	—	—
5	1 000	160	6 75	52	0 18
6	10 000	200	8 99	—	—

* A growth hormone preparation containing ACTH contaminant

constituents seem very much lower than one might expect In rats for example the ascorbic acid content of the whole gland ranges from 300 to 500 mg per cent and the adrenal cholesterol content ranges from 2 to 4 per cent (6) With bovine glands obtained at slaughter we have only a fraction of these values The cholesterol in these isolated beef glands has been subjected to fractionation with digitonin and it turns out that 90 per cent of the total is free while only 10 per cent is combined with lipoid

There is a simple explanation for this apparently abnormal situation The adrenals we have been working with are obtained from cows which had been stunned by a blow on the head exsanguinated skinned and finally eviscerated A rather significant time interval thirty minutes or so thus elapses before we are able to remove the gland from the carcass It seems quite likely that the bulk of the ester cholesterol and ascorbic acid initially present in the gland were almost completely removed during the severe stress associated with the slaughtering procedure Thus we start our perfusion studies with glands that are more or less exhausted with respect to both cholesterol and ascorbic acid In addition to this unphysiologic beginning it is only fair to state that these adrenal glands are perfused with unphysiologic media under unphysiologic conditions The blood employed for perfusion for example contains citrate as an anticoagulant the glucose concentration is perhaps ten times greater than normal and the plasma potassium values are abnormally high As our perfusion technique has evolved

need not necessarily be associated with steroidogenesis This conclusion drawn from *in vitro* data happens to be in agreement with experiments on intact animals reported by several groups* (7) In view of this I should like to raise the question as to what role ascorbic acid plays in adrenal cortical metabolism

Ralli You are indicating that the decrease in the adrenal ascorbic acid is independent of the synthesis of the steroid hormones?

Hechter I did not say that I said cortical hormones may be synthesized without necessarily having a drop in adrenal ascorbic acid

Jensen What is your proof for that? The adrenal may take up cholesterol or ascorbic acid from your perfusion fluid You first have to determine the balance of cholesterol and ascorbic acid between the gland and the perfusion fluid Simply because you don t get any change in the gland *per se* is no proof that there is no change

Hechter The blood used for perfusion is stored sometimes for six or seven days at 2° C On numerous occasions we have attempted to measure the ascorbic acid content of such stored blood but we have not found measurable amounts In other experiments we have added varying amounts of ascorbic acid (sometimes as much as 1 gm per liter) to perfusion blood and have not materially affected the biosynthesis picture with or without added ACTH These observations taken together lead us to the view that steroidogenesis in isolated perfused adrenal glands can occur without the recognizable participation of vitamin C

Long I agree with that conclusion Dr Hechter but it seems to me that an animal such as the cow which has been synthesizing ascorbic acid presumably in the adrenal may have ample precursors for the synthesis in the perfused blood without ascorbic acid necessarily being there

Pitts Have you ever perfused these adrenal glands with some artificial medium?

Hechter Yes we have

Pitts Do you get synthesis of steroid?

Hechter For ACTH induced steroidogenesis we seem to need blood

Thorn Dr Long s point would be demonstrated by an appreciable increase in blood ascorbic acid in the perfusate as it left the adrenal gland wouldn t it?

Long Martha Vogt was not able to find any change in the ascorbic acid content of the blood draining the gland at a time when one presumes the ascorbic acid within the gland was disappearing

Pincus You could take exactly the opposite point of view and say

---

E N JI N F S o R d PINCUS G A t 1 n p p at on

Vollmer In the hamster adrenal there is practically no cholesterol to begin with Until you have some normal values for the cow, it is a little hard to insist that your perfusion adrenals were initially depleted, although you are probably right

Hechter I am not insisting

Long There is practically no cholesterol in the adrenal of the hamster?

Vollmer It is considered lipid poor They found at Rutgers that hamsters infected with Leishmania tended to build up their cholesterol, however Dr J H Leathem mentioned that at the Laurentian Hormone Conference

Long Do you know the cholesterol values?

Vollmer I have seen it reported at zero, at least histochemically

White This raises the interesting corollary problem which a number of people have thought about namely the variation of ACTH in pituitaries of various species Is this related at all to the degree of struggling which the animal exhibits during slaughtering or is this a real species difference?

Hechter I should like to have someone nembutalize a cow and remove the adrenals for analysis of tissue constituents

Bloch Have you analyzed the blood of these cows that have been stunned? If so much steroid is poured out, do you think that it is as cholesterol or as steroid hormone?

Hechter I pointed out earlier that in some cases sizable amounts of a variety of steroids may be isolated from the blood of cows I mentioned that this is a variable picture and it is not unlikely that the results we have obtained with glands secured in Worcester may differ in a number of important respects from the results obtained by some one say in Chicago who has access to glands removed in an efficient standardized slaughterhouse procedure

Bloch It seems to me that if you took a blood sample from a living cow in contrast to taking the blood from a slaughtered animal you would find very marked differences if this kind of decline in adrenal cholesterol represents output of hormones

Hechter We have not drawn blood samples from bovines although this comparison you mention would seem well worth doing Nor do I know whether we are actually losing 90 per cent of the adrenal cholesterol The absolute loss involved seems to me to be a secondary question What does seem important is this granted that we start with a preparation which seems to be grossly abnormal there is no decline in adrenal ascorbic acid associated with biosynthesis and release of corticosteroids in these glands This might suggest that the drop in adrenal ascorbic acid content which one uses to assay ACTH activity

Hechter This has been repeated on about ten blood samples I have indicated previously that the results were quite variable but in some cases the patterns exhibited in Table XII were fully reproduced The blood used in these experiments was obtained from animals killed by kosher methods or otherwise In kosher slaughter the neck vessels are severed in a single thrust and the blood obtained is a mixture of carotid arterial and jugular venous blood In non kosher killing the blood obtained represents principally carotid arterial blood

White Dr Long on this question of ascorbic acid didn t the experiments which you and Miss Osterling carried out on the scorbutic guinea pig and the subsequent studies of others show that the scorbutic guinea pig having little vitamin C reserve could discharge adrenal cortical steroids following ACTH?

Long Yes

White What is the interpretation of those studies in relation to ascorbic acid in steroidogenesis? Does this weaken the general hypothesis that ascorbic acid is involved?

Long To our minds it does not remove the fact that ascorbic acid if present in some way participates in the series of events which follow stimulation of the gland with ACTH On the other hand as far as the results with guinea pigs go and they were very decisive it is not an obligatory transformation There are other materials in the gland which can take over the function of ascorbic acid when it is absent Of course it should be re examined on a more quantitative basis but so far as we could tell the response of the adrenal gland of the guinea pig after fourteen days on a scorbutic diet as judged by the lymphocyte and cholesterol change was about the same as in the animals receiving ascorbic acid in their diet

Astwood Dr Hechter you compared your system with the tissue homogenate systems Has anyone shown in the latter that corticotropin will cause an increased rate of production of sterols?

Hechter I compared perfusion to tissue slice and homogenate systems only in the sense that all of these represent *in vitro* metabolic systems in which the results obtained must be viewed critically and they all suffer from the same limitations when one attempts to assess their physiologic significance I know of no experiments in which ACTH has been shown to have effects in homogenate systems However Haines *et al* (8) and Zaffaroni * working independently have incubated adrenal slices with ACTH Zaffaroni has informed me that he was never able to obtain a net increase in the total amount of corticosteroid by such incubations Haines and his co workers however have demonstrated that radioactive 17 hydroxycorticosterone and other corticoste

* Zaffaroni A. Personal communication

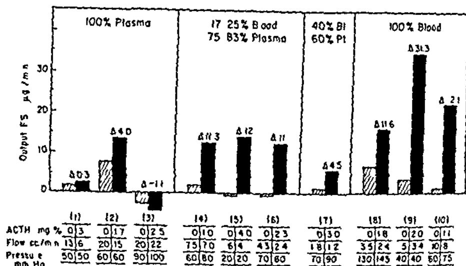


FIGURE 30 The results of ten experiments wherein bovine glands were perfused with plasma plasma blood mixtures and blood in the absence and presence of ACTH Reprinted by permission from Hechter O *et al* Nature and biogenesis of adrenal secretory product *Recent Progr Hormone Research* 6 215 (1951)

that ascorbic acid is inhibitory to steroidogenesis in the gland and its decrease is necessary for steroidogenesis

Hechter Figure 30 will answer Dr Pitts question in part Here we have prefused glands with whole blood blood diluted with plasma and with plasma alone In all cases the glands were perfused for an initial period without ACTH and for a second period with ACTH It is evident that in only one of the three experiments was ACTH effective in increasing corticosteroid output when plasma was employed as the perfusion medium With whole blood the ACTH effect was consistently observed and this still occurs when as little as 20 per cent blood is used in conjunction with plasma

Long That means you need oxygen for the synthesis of adrenal steroids doesn t it?

Hechter That is one explanation Plasma has sufficient oxygen carrying capacity however for the 11 hydroxylation of desoxycorticosterone This conversion proceeds almost as well in plasma and in artificial media not containing hemoglobin as it does in blood We do not know whether red cells are necessary because of their oxygen carrying capacity or because they contain a cofactor for the biosynthetic process stimuated by ACTH

Nelson I should like to ask about the levels which you find in non perfused blood They seem rather high on the basis of what we have found Have you repeated this on a number of occasions and from what vessel was this blood obtained?

Hechter This has been repeated on about ten blood samples I have indicated previously that the results were quite variable but in some cases the patterns exhibited in Table XII were fully reproduced The blood used in these experiments was obtained from animals killed by kosher methods or otherwise In kosher slaughter the neck vessels are severed in a single thrust and the blood obtained is a mixture of carotid arterial and jugular venous blood In non kosher killing the blood obtained represents principally carotid arterial blood

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* ZAFFARONI A Personal communication.

roids are formed when adrenal slices, but not homogenates are incubated with C^{14} labeled acetate in the presence of ACTH Results in this system in the absence of added ACTH were not reported Thus it would seem considering Zaffaroni s work in conjunction with that of Haines and his co workers that whatever synthesis does occur in slices is balanced by destruction so that there is no net increase of corticosteroid

Astwood I was thinking that if corticotropin does affect your system and not adrenal slices or homogenates it would imply that yours is the more physiologic system

Hechter I would not disagree with the view that perfusion is more physiologic than slices I think it is However I should like to point out that there is a great difference between slices and the perfusion technique in a biochemical sense aside from the question of organization In a slice or homogenate the products of reaction accumulate in the system where they may possibly inhibit the rate or alter the course of reactions In a perfusion system however the products of reaction may be removed from the system Perhaps this feature is more important than whether one uses slices or a perfused organ

Loewi Did you say that one of these components was also found with serum in the absence of oxygen or without blood present? Which compound was it? I understood that you got synthesis of one steroid with serum or plasma alone

Hechter Desoxycorticosterone may be converted to corticosterone by adrenal perfusion in the absence of red cells One may use plasma or a variety of simple colloidal solutions and obtain this reaction in isolated perfused glands

Loewi With desoxycorticosterone?

Hechter Yes

Loewi But with none of the others?

Hechter I did not say that

Pincus We have not tried the others with artificial media to any extent

Li Do you have some information on how small a dose of ACTH one can use and still achieve a significant effect? The lowest dose which you mention is 1 mg Have you tried it in the range of micrograms?

I should also like to ask whether you have identified any androgenic steroids after perfusion with ACTH A question which has been bothering us concerns the secretion of androgenic steroids by animals stimulated by ACTH injection In our *in vivo* studies with ACTH no matter how large a dose we have injected into rats we have so far found no stimulation of the testes or of the ovaries and the gonads in general So I am wondering whether in your study of adrenal perfusates you have

found any indication of an effect of ACTH on androgen production

And finally, what do you estimate is the percentage of ACTH in the growth hormone you have been using?

Hechter To answer your second question first we have examined the possibility that isolated perfused glands release androgens We have also searched for estrogens This work by Dr Pincus Dr Dorfman and myself has engaged our attention for the last six months Unfortunately we cannot answer your question since the results obtained are quite variable Sometimes (and this happened three out of ten times) a perfused gland will give clear evidence of the release of androgenic activity as assayed on the chick comb In the other cases however there was no effect A similar situation obtains with estrogens We get in a small number of experiments very definite evidence for the output of estrogenic activity but here again the evidence is quite variable More data is obviously needed

White May I make the comment that perhaps one should distinguish in connection with the examination of adrenal perfusates whether one is talking about androgenic biological activity or whether one is talking about C 19 steroids because 17 hydroxyprogesterone for instance exhibits significant androgenic activity I think perhaps Dr Pincus and Dr Hechter would agree that one would have to have definite chemical characterization or at least rather quantitative comparative biological assays Is that a fair statement Dr Pincus?

Pincus It is exactly what I had in mind

Hechter Clearly more must be done than just a determination of androgenic activity We simply started that way We plan to proceed using paper chromatography with an attempt to determine whether C 19 steroids are actually elaborated by adrenal glands

Before I answer Dr Li s first and third questions a word of explanation Sometime ago Dr Li sent us a sample of one of his growth hormone preparations which he regarded as homogeneous In our hands this material showed definite activity both in the Munson test (9) and upon the isolated perfused adrenal We informed Dr Li of our findings and subsequently at several meetings have made the statement that Li s growth hormone tested by us contains an ACTH contaminant Last evening Dr Li Dr Pincus and I had a discussion in which Dr Li informed us that he has tested this same preparation for ACTH contamination by the Sayer s method (10) and found absolutely no adrenal ascorbic acid depletion activity despite the administration of mg doses As a result of this three way discussion all of us are now convinced that there was indeed an ACTH contaminant in the growth hormone preparation we worked with but we are not certain where it was introduced whether in Worcester or elsewhere It is possible for example as Dr

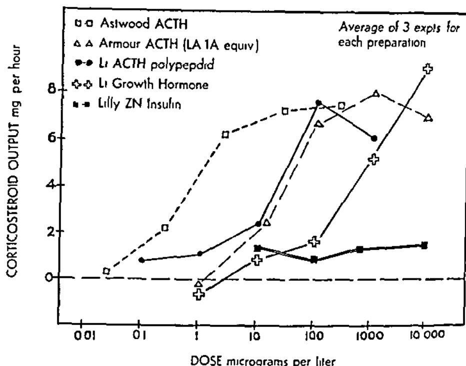


FIGURE 31 Comparative ACTH activity of various hormonal proteins

Li pointed out that some ACTH incompletely removed from our glass ware might have contaminated his growth hormone preparation In which case the discrepancy between our results would be resolved In any event Dr Pincus and I are now convinced that this whole question must be re examined

To answer Dr Li s question concerning the minimal dosage of ACTH necessary to stimulate the perfused adrenal may I present Figure 31 This illustrates the log dosage response curves obtained with three ACTH preparations the growth hormone preparation obtained from Dr Li and a highly purified sample of insulin Each experiment was done in the following manner At a constant flow rate of 1 liter per hour blood was perfused through a single gland the perfusate being collected without being recirculated Successively increasing concentrations of hormonal protein were added to the blood and the content of formaldehydogenic steroid measured The curve shown represents the mean of three different experiments with each preparation Four of these preparations increased the corticosteroid output from isolated glands All show a sigmoid log dosage response curve the slopes of which are remarkably similar The most active preparation tested in the series is the Astwood ACTH preparation The Li ACTH polypep

tide is somewhat more active than the LA 1 A standard The growth hormone preparation is the least active and the insulin had no effect in the dosage tested

Bauer Is that all on the same adrenal?

Hechter No three different experiments with each hormonal protein preparation

Long *Have you tried any other growth hormone preparation* for instance the one made by the Fishman Wilhelmi procedure?

Hechter In the isolated gland? No

Li Dr Long this preparation is actually a combination of our method and the Armour procedure and it has been tested by a number of biological assays for thyrotropic gonadotropic ACTH and other contaminants

Hechter These five preparations were assayed by the Munson modification (9) of Sayer s method (10) and the results compared to those obtained with the perfused adrenal Table XIV shows these results arbitrarily setting the LA 1 A standard as 1 The comparative results using these dissimilar methods illustrate that the agreement is not too bad

Our findings with Li s growth hormone preparation indicate that the degree of contamination is 2 per cent (of the activity of LA 1 A) by the Munson test and 3 per cent on the basis of the perfused adrenal If one accepts the fact that material at least one hundred times the potency of LA 1 A may be obtained which is still not homogeneous (11) the degree of contamination might be of the order of 0 02 per cent However Dr Li tells us *that in his hands this growth hormone* preparation is completely devoid of activity in the adrenal ascorbic acid test and that a battery of physicochemical and biological tests which he has employed has led him to the conclusion that his preparation is homogeneous Dr Pincus and I wish we had more of this growth hor

TABLE XIV

Comparative Activity of Preparations Assayed by Ascorbic Depletion Method and by Corticosteroid Released from Perfused Bovine Adrenal

Preparation	Activity relative to Armour LA IA	
	Munson method	Perfused adrenal
Armour ACTH	1 0	1 0
Astwood ACTH	105 0	40 0
Li ACTH ide	1 5	1 3
Li Growth hormone	0 020	0 034

mone preparation Dr Li, so that we and possibly some third laboratory might retest the question of ACTH contamination

Li By the ascorbic acid depletion test?

Hechter Yes just by one test We feel certain that our results with growth hormone are due to an ACTH contaminant and not to the growth hormone moiety because when this material was boiled for one hour at pH 1 all of the ascorbic acid depleting activity was retained I feel certain that you would agree that the growth hormone probably would not withstand that type of treatment

Li It would be destroyed

Hechter May we proceed to the next question?

It has been made clear that the perfused bovine adrenal preparation treated with ACTH, is an *in vitro* system which can synthesize a corticosteroid mixture the principal components of which are corticosterone and 17 hydroxycorticosterone Are these corticosteroids actually derived from cholesterol as has been generally believed albeit on indirect evidence? We thought we might be able to answer that question in a definitive fashion by perfusing labeled cholesterol through adrenal glands Since adrenal slices (12) like liver (13) have the ability to condense C^{14} labeled acetate to form radioactive cholesterol we decided it would be interesting to perfuse radioactive acetate as well through the adrenal in the presence of ACTH

C^{14} cholesterol labeled in position 3 (obtained from Dr Schwenk)

TABLE XV

The Specific Activities of Cortical Hormones and Their Derivatives Isolated from an Adrenal Perfusion Experiment with $CH_3C^{14}OONa$

	Compounds	mg	c/m/mM (× 10⁴)
1	Compound F	125	3 52
2	Compound F after rechromatography	115	3 79
3	Compound F acetate	130	4 00
4	Compound F propionate	098	3 66
5	Compound E acetate	145	3 73
6	Adrenosterone	110	3 60
1	Compound B	130	3 22
2	Compound B after rechromatography	090	3 44
3	Compound B acetate	123	3 10
4	Compound B propionate	110	3 50

and carboxyl labeled sodium acetate were employed in these experiments A group of five glands were perfused through a manifold with 1 to 4 liters of blood containing 25 mg ACTH (Armour) for four hours the perfusate being continually recycled At the end of the experiment the blood perfusate was worked up for corticosteroids and cholesterol and the perfused tissue was extracted for cholesterol 17 hydroxycorticosterone and corticosterone were purified and isolated by paper chromatography the cholesterol in blood and adrenal tissue was fractionated by silica gel chromatography into free and combined fractions Thus in each experiment there were four cholesterol fractions These were worked up separately and were purified by saponification chromatography on silica gel repeated crystallization and formation and chromatography of derivatives Four experiments were performed (two with radioactive acetate two with labeled cholesterol)

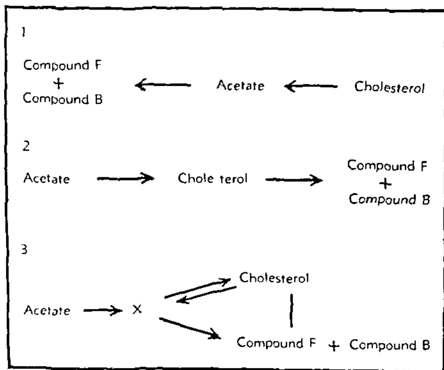


FIGURE 32 Possible relationships between acetate cholesterol and corticosteroids

and in all cases the corticosterone and 17 hydroxycorticosterone isolated were radioactive The ratio of the count of corticosterone to 17 hydroxy corticosterone in each experiment was in the neighborhood of 1 That the 17 hydroxycorticosterone and corticosterone isolated actually repre sent radioactive steroid is shown in Table XV It will be seen that the *count remains constant through a variety of derivatives We therefore* feel that our counts for the corticosteroids represent meaningful values

Up to this point we have shown that 17 hydroxycorticosterone and corticosterone may arise from either acetate or cholesterol Figure 32 illustrates three possible relationships between acetate cholesterol and *corticosteroids The first is that acetate condensation to form corti* costeroid involves cholesterol as an obligatory intermediary A second possibility somewhat unlikely is that corticosteroids are formed exclu sively from the condensation of 2-carbon fragments On this basis cholesterol transformation to corticosteroid would not involve mere *side chain degradation but would require deep seated changes in the* steroid nucleus with the formation of acetate as an obligatory inter mediary Finally a third possibility would be one wherein acetate and cholesterol act as precursors through alternative pathways This would involve the situation where acetate conversion to corticosteroid need *not involve cholesterol as an obligatory intermediary and where cho* lesterol transformation does not involve acetate as an obligatory in termediary In this situation one might envisage as a likely possibility the condensation of 2 carbon fragments arising from acetate to a sub stance which we may designate as X which has the capacity of being *converted to either cholesterol or corticosteroid*

Table XVI presents a comparison of the radioactivity of the corti costeroids relative to the various cholesterol fractions obtained at the end of the experiment In these experiments the radioactivity is ex pressed in terms of counts per minute per millimol

In all experiments whether cholesterol or acetate was used as the C 14 substrate the cholesterol fraction exhibiting the highest specific activity uniformly proved to be the adrenal free cholesterol The combined *cholesterol fractions whether from adrenal or blood were low sug* gesting that the esterification of cholesterol with lipoid did not proceed rapidly in this system If the compounds F and B arising from acetate were required to pass through cholesterol as an obligatory intermediary the count of the compounds F and B arising from the cholesterol pool should be *no greater* than the count of the adrenal free cholesterol This should follow if the reaction of cholesterol formation and degra dation to corticosteroid are close in time We have previously shown that ACTH acts within thirty seconds upon *the isolated adrenal* (1) The finding therefore that compounds F and B have a count 6 4 times

TABLE XVI

C 14 Substrate	Isolated Cholesterol			Isolated Corticoids			Ratio
	Fraction	Experiment		Fraction	Experiment		Corticoids / Adrenal free
		No 1 c/m/mM ($\times 10^5$)	No 2		No 1 c/m/mM	No 2	
Acetate	Adrenal free	3 69	4 32	Compound F	37 5	16 9	6 4
	Adrenal combined	0 53	1 30				
	Blood free	1 42	2 17	Compound B	33 1	14 4	
	Blood combined	0 20	0 13				
Cholesterol	Adrenal free	7 35	3 34	Compound F	3 50	1 04	0 40
	Adrenal combined	0 58	0 34				
	Blood free	2 28	0 52	Compound B	2 46	0 96	
	Blood combined	0 22	0 13				

TABLE XVII

C 14 Substrate	Experiment No	Total counts added	Total counts recovered as B F	% Conversion	Average
Acetate	1	$6\ 9 \times 10^{8}$	$3\ 9 \times 10^{4}$	0 006	0 005
	2	$6\ 9 \times 10^{8}$	$2\ 8 \times 10^{4}$	0 004	
Cholesterol	1	$1\ 44 \times 10^{6}$	$1\ 25 \times 10^{3}$	0 10	0 300
	2	$1\ 60 \times 10^{6}$	$7\ 97 \times 10^{3}$	0 50	

greater than that of the adrenal free cholesterol suggests that cholesterol need not be an obligatory intermediary in the synthesis arising from acetate

In Table XVII the same experiments are listed this time in terms of the percentage of conversion of added radioactivity into corticosteroid It will be noted that cholesterol is 60 times more efficient than acetate as a precursor This must mean that cholesterol cannot go to corticosteroid exclusively via 2 carbon fragments because had this been the case cholesterol should have the same order of efficiency as acetate

This takes us back to Figure 32 where we may now discard possibilities one and two We are left with alternative pathways for corticosteroid synthesis This is not an uncomfortable position It is rather reasonable to have a gland which can condense 2-carbon fragments to form a C 27 structure be able to stop the condensation process at a C 21 stage And similarly it is reasonable for a gland which must respond within seconds to emergency situations by pouring out corticosteroid to have a storehouse of steroid precursor available for immediate transformation to cortical hormone The cholesterol acetate situation in corticosteroid metabolism might be likened to carbohydrate metabolism where the cholesterol would be analogous to glycogen and the acetate to glucose This is a picture where 2 carbon fragments from acetate and cholesterol work together it is not an either/or hypothesis

Loeb In which form did you use cholesterol? To my knowledge it is poorly soluble in plasma Thus I do not quite understand how you worked with it

Hechter I am certain that the low efficiency of radioactive cholesterol as a precursor which we observed is intimately related to the poor solubility A colloidal suspension of cholesterol was prepared and added to blood Once it was mixed however we really do not know what happened I would not be surprised if a considerable amount of the

added cholesterol were not effectively circulated through the glands But I would point out that even under these adverse circumstances we were able unequivocally to demonstrate the transformation of cholesterol to cortical hormone

There is one other point arising from these findings which should be mentioned that is that blood cholesterol as such may be utilized by adrenal glands for the synthesis of corticosteroid

Bloch The values for blood cholesterol in the acetate experiments were lower than the gland cholesterol Wouldn t that indicate that the preferred source is the tissue cholesterol rather than the blood cholesterol?

Hechter Yes

Bloch I think the third of your schemes is the most plausible one A great deal depends on whether you are thinking of free cholesterol or of combined forms The fact that the ester cholesterol has a much lower activity than free cholesterol indicates that isotopic equilibration had not occurred You may have combined forms of cholesterol which are more active than the free sterol The second possibility is that a close derivative such as cholestenone might go to cholesterol as well as to cortical hormones I should like to ask whether you still feel as Dr Pincus suggested last year that the point of action of ACTH might be in the removal of part of the cholesterol side chain?

Hechter I think that goes to the heart of what may be a very important question Perhaps our data is not incompatible with the notion that cholesterol is an obligatory intermediate in the synthesis from acetate to corticosteroid As you correctly point out the concept of an isotopic pool requires that you have equilibrium conditions Here we are clearly not at equilibrium because you will have noticed there are profound differences in the specific activities of the various cholesterol fractions If you assume however that the cholesterol which is isolated as free cholesterol from adrenal tissue enters a free cholesterol pool and that this pool is homogeneous you come out with the notion that cholesterol need not be an obligatory intermediary in the synthesis of cortical hormones from C 2 fragments If it is assumed as Dr Bloch suggested that a closely related substance like cholesterone might be involved then one has a new compound not cholesterol It would become analogous to the X in our scheme Finally if there is a form of cholesterol in the adrenal which has the potentiality for going to corticosteroid at a rate faster than the ordinary cholesterol in the gland then it is not the ordinary adrenal cholesterol but some active form The situation might be one as Dr Bloch inferred wherein there is an acetate and an active acetate But no matter how it is looked at one is left with the notion that if acetate condenses to something say X this

X cannot be the ordinary cholesterol that we extract as free cholesterol in adrenal tissue

I should like to ask Dr Bloch what information he could advance on the subject of acetate condensation giving a steroid nucleus We would like to think of acetate condensing to something like a C 19 structure a very reactive C 19 substance having a steroid nucleus which under appropriate circumstances condenses with metabolites of one type or another to give various side chains This is pure speculation But I should like to know whether he could give us some information as to what goes on between C 2 and C X

Long That is a large order Dr Bloch Do you wish to undertake it?

Bloch There is just one possibility which might permit the direct and indirect pathway to be decided Suppose you incubate or perfuse in the presence of labeled acetate before adding ACTH for an hour or so In that way you would build up the isotope content of cholesterol in the tissue Then you add ACTH In this case it seems to me the two steroids should become closer in isotope concentration

Hechter An experiment of that type might be very interesting We have done a single experiment in another direction which may be significant We have very slowly perfused nonradioactive dehydroepiandrosterone together with carboxyl labeled acetate through a gland to determine whether or not the C 2 fragment might condense on the C 19 steroid to give us a C 21 corticosteroid From this perfusate a small amount of compound F was obtained This compound F counted at about 600 per minute per mg Now if the C 2 fragments merely condensed with other C 2 fragments to give rise to a C 21 steroid the count should remain approximately the same following removal of the side chain If however there was indeed a condensation of a C 2 on a C 19 acceptor lopping off the side chain should markedly reduce the radioactivity of the resulting steroid This compound F was subjected to chromic acid oxidation and the product obtained counted about 60 per minute per mg

I should like to emphasize that that is only one experiment and not a very good experiment for several reasons which I shall not go into However it suggests the possibility that you may have condensation on a reactive C 19 structure of which dehydroepiandrosterone might represent a stable form

Conn I should like to bring up a point Two years ago at this Conference we presented some gross clinical observations which led us to suggest that esterified choloesterol of the blood might be used by the adrenal cortex for the production of adrenal steroids when the gland in man was pushed hard by ACTH over a period of time We showed that the cholesterol content of the serum did not fall until about the third

or fourth day of ACTH administration and that then it dropped precipitously We suggested that at this time there arose the need of another source of precursors of adrenal steroids that adrenal cortical production of cholesterol could not keep pace with the rate of steroid hormone production And inasmuch as the serum esterified cholesterol fell very sharply and since the adrenal gland normally contains about 90 per cent of its cholesterol in the ester form it was suggested that perhaps ester cholesterol of serum is a precursor of adrenal cortical hormones when the adrenals are forced to produce large amounts of hormone for long periods of time

In Dr Hechter s demonstration here you recall that on the basis of the initial analyses of the adrenal glands before perfusion he indicated that they are relatively worn out that they contain essentially no esterified cholesterol In the experiments in which the labeled acetate or cholesterol was added it was found that essentially all of the labeling was in the free cholesterol and not in the combined fraction But it should be pointed out that the gland contained little or no ester cholesterol to begin with

Dr Hechter also pointed out that one must not regard these perfusion studies as physiologic experiments I wonder whether these experiments eliminate the possibility that esterified cholesterol is an important precursor in the physiological state

Hechter Our data do not eliminate the possibility They raise however the alternative possibility that free cholesterol may be the principal corticosteroid precursor not only in the isolated adrenal system but in man as well The findings which Dr Conn described would then be explicable in terms of free cholesterol uptake by the gland and its replacement from the ester cholesterol compartment This would operate to maintain the free cholesterol of the blood plasma at the expense of the ester cholesterol Further work would be necessary to decide between these alternatives

I should like to go on if I may At this point we know that 17 hydroxycorticosterone and corticosterone are the primary end products of corticosteroid biosynthesis in the isolated adrenal gland Evidence has been presented that they can arise from cholesterol and acetate presumably through alternative pathways What are the steps in between?

To answer this question we thought it would be useful to perfuse a variety of steroids through isolated glands in order to evaluate the enzymatic capacities of adrenal tissue Given this data it might be possible to put it together to form a scheme of the metabolic pathways involved in the conversion of cholesterol to adrenocortical hormones Figure 33 illustrates our scheme It is important to mention that this

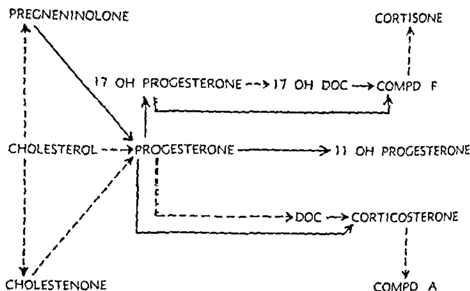


FIGURE 33 Tentative scheme of corticosteroidogenesis wherein cholesterol is assumed to be the primary steroid precursor The solid lines represent reactions which have been demonstrated the dotted lines represent possible reactions which have not as yet been demonstrated Reprinted by permission from Hechter O *et al* Nature and biogenesis of adrenal secretory product *Recent Progr Hormone Research* 6 215 (1951)

may well be modified as further data appear and that it does not represent a final and definitive picture The conversions definitely established to date are indicated by solid lines those not proved but which seem potentially likely by dotted lines It may be seen that we envisage as the first step a degradation of the cholesterol side chain to yield pregneninolone the 3 β hydroxyl function of pregneninolone is then oxidized to form the Δ 4 3 keto steroid progesterone

Alternatively one may envisage that the conversion of cholesterol to progesterone involves cholesterone as an intermediary thus excluding pregneninolone but there is no evidence for or against this view To settle this point we hope to perfuse radioactive cholesterone From progesterone a dual series of transformations is suggested one leading to corticosterone as an end product the other to 17 hydroxycorticosterone Pregneninolone and progesterone are included as intermediates because it has been definitely established that perfusion of these steroids through adrenals leads to the formation of both corticosterone and 17 hydroxycorticosterone By perfusion of pregneninolone we have found that in a single cycle pregesterone is the major product 17 hydroxy corticosterone and corticosterone becoming the major products only

upon recycling the perfusate

Please note that in the transformations from progesterone there is a series of 17 hydroxylated steroids on the one hand and a series of 17 desoxy steroids on the other Evidence has previously been presented for the existence of these dual pathways (1) Briefly stated this consists of the finding that neither 11 desoxycorticosterone nor corticosterone can be 17 hydroxylated by perfused adrenals tested under a variety of conditions (including ACTH ascorbic acid vitamin mixtures etc) to yield 17 hydroxycorticosterone Only when the 17 hydroxyl function was present in the corticosteroid molecule (i e with Reichstein s S) was it possible to obtain 17 hydroxycorticosterone on adrenal perfusion The possibility was then considered that the C 21 hydroxyl function in corticosteroids inhibited the introduction of oxygen in the 17 position Progesterone was perfused with the results already described 17 hydroxyprogesterone has been clearly identified as a transformation product in adrenal perfusates with progesterone Upon perfusion of 17 hydroxyprogesterone to date only 17 hydroxycorticosterone and no corticosterone has resulted The conversion of progesterone to corticosterone may involve DCA or 11 hydroxyprogesterone the latter but not the former having been isolated from progesterone perfusates Recent studies by Drs Andre Meyer and Roger Jeanloz indicate however that 11 hydroxyprogesterone is not converted to any appreciable extent by adrenal perfusion to either 17 hydroxycorticosterone or corticosterone These observations therefore strongly suggest that desoxycorticosterone is the intermediary its absence in perfusates involving progesterone being due to its rapid transformation to corticosterone The conversion of progesterone to 17 hydroxycorticosterone may go via (*a*) 17 hydroxyprogesterone to 17 hydroxy 11 desoxycorticosterone (*b*) 17 hydroxyprogesterone to 11 17 dehydroxyprogesterone or (*c*) 11 hydroxyprogesterone to 11 17 dehydroxyprogesterone The last possibility seems unlikely in view of the previously mentioned finding that adrenal perfusion of 11 hydroxyprogesterone does not lead to significant amounts of 17 hydroxycorticosterone Since the perfused adrenal appears to have a limited capacity to transform the 11 hydroxylated steroids which have been perfused through the system (namely corticosterone 17 hydroxycorticosterone and 11 hydroxyprogesterone) it may well be that once the 11 hydroxyl group is introduced into the steroid nucleus it becomes an end product of adrenal cortical metabolism and is no longer acted upon by adrenal enzymes If this be true then progesterone transformation to 17 hydroxycorticosterone involving 11 17 dehydroxyprogesterone is excluded Cortisone and dehydrocorticosterone are shown in Figure 33 as secondary derivatives of 17 hydroxycorticosterone and corticosterone Please note however that these reac

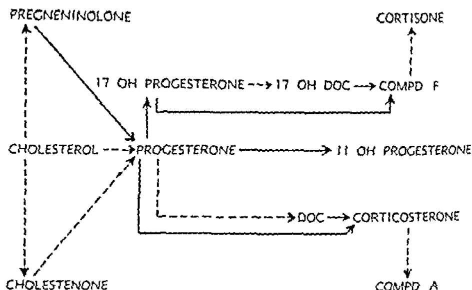


FIGURE 33 Tentative scheme of corticosteroidogenesis wherein cholesterol is assumed to be the primary steroid precursor The solid lines represent reactions which have been demonstrated the dotted lines represent possible reactions which have not as yet been demonstrated Reprinted by permission from Hechter O *et al* Nature and biogenesis of adrenal secretory product *Recent Progr Hormone Research* 6 215 (1951)

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composition of these adrenal oxidase systems the role of ascorbic acid or of pantothenic acid in adrenal cortical metabolism may well be clarified

The reaction wherein pregneninolone is oxidized to progesterone has been shown in adrenal slices but this reaction it should be emphasized proceeds in other steroidogenic organs (19) as well I was wondering Dr Nelson whether Dr Samuels group has been able to show preg neninolone conversion to progesterone in adrenal homogenates

Nelson They have only used slices in their work I don t think they have tried homogenates

Hechter Finally I should mention that the degradation of C 27 cholesterol to form C 21 corticosteroids presumably involving preg neninolone as a primary intermediary has been observed to date only in perfused adrenal tissue and it has not as yet been achieved with either adrenal homogenates or slices

Given this scheme of steroidogenesis the question arises as to which step or steps in this series of reactions is regulated by ACTH The an swer to this question seems important because it is widely held that the mechanism of hormone action at a molecular level will ultimately find explanation in terms of hormonal regulation of one or more tissue en zyme systems This view first presented formally by David Green (20) in a general theory to account for the profound physiological effects of a variety of agents which act at extremely dilute concentration (vitamins certain drugs and toxins as well as hormones) carries implicitly with in it the idea that the specificity of hormone action is the result of the interaction of particular hormones with specific enzyme systems

Table XVIII represents a tentative approach to the localization of ACTH action in the corticosteroidogenic reactions leading from cho lesterol Here will be seen a comparison of the rates at which various steroid substrates give rise to compounds F and B in perfused bovine glands First it will be seen that with perfusion with blood in this sys tem little or no corticosterone and 17 hydroxycorticosterone is formed in the absence of ACTH Upon the addition of ACTH to the blood 11 hydroxy steroids are formed at a rate of 1 to 2 mg per hour This indicates that ACTH stimulates a reaction in the system which barely proceeds in its absence As a first approximation this might suggest that any reaction which goes in the absence of ACTH is not a likely point of ACTH action From Table XVIII it is evident that desoxy corticosterone 17 hydroxydesoxycorticosterone and progesterone and pregneninolone all in the absence of ACTH give rise to 11 hydroxy corticosteroid at rates greatly exceeding those obtained with ACTH Where ACTH has been tested namely on the 11 hydroxylation reac tion it did not influence the rate We have not as yet tested the in

tions have not been demonstrated directly Their presence in ACTH perfusates although in small amount does suggest the presence of an adrenal system which oxidizes the 11 hydroxyl to an 11 keto group

If one assumes that such a scheme of steroidogenesis mirrors the *in situ* situation a number of interesting implications arise This scheme would suggest that there need not necessarily be certain adrenal cortical hormones (e g , 17 hydroxycorticosterone and corticosterone), but that depending upon conditions other steroids might become the predominant hormones released For example it is possible that disease or chronic stress might more or less selectively influence certain of the oxidase systems described If the C 11 oxidase system was selectively blocked the end products of corticosteroidogenesis might be desoxycorticosterone and 17 hydroxy 11 desoxycorticosterone If the 17 hydroxylation is interfered with then corticosterone and desoxycorticosterone might be the end products of corticosteroid metabolism in such a situation As you can see this scheme permits a great number of possibilities for qualitative changes in the character of the adrenal secretory product

Many of the reactions first discovered with perfused adrenals have been confirmed in simpler *in vitro* systems The 11 hydroxylation reaction in homogenates (14 15 16) with both desoxycorticosterone and 17 hydroxydesoxycorticosterone as substrates has been intensively studied and the system is beginning to be fairly well characterized The oxidase system appears to be associated with the heavy particles in homogenates perhaps derived from mitochondria (14 17) and the necessary cofactors are being elucidated In a similar fashion the C 21 oxidase system has been under investigation in several laboratories Recently Drs Hayano and Dorfman (18) at the Worcester Foundation have demonstrated the 21 hydroxylation of progesterone 17 hydroxy progesterone and 21 desoxycortisone in adrenal homogenates the products formed being corticosterone 17 hydroxycorticosterone and cortisone respectively Since progesterone transformation to 17 hydroxycorticosterone in homogenates has not been achieved as yet (although it has been the subject of intensive investigation) the step from progesterone to 17 hydroxyprogesterone remains to be demonstrated It seems reasonable to expect that with further study of cofactors and conditions this reaction will likewise be achieved in adrenal homogenates If one is interested in studying the reactions of progesterone or 17 hydroxyprogesterone to corticosterone or 17 hydroxycorticosterone it seems clear that homogenates and enzyme extracts represent the best tool for the elucidation of these systems For most questions in this field perfusion has probably outlived its usefulness now that simpler systems are available In the course of elucidating the nature and

were so the concept of the qualitative specificity of pituitary tropic hormone activity would be seriously shaken

If one wishes to retain the specificity of tropic hormone action a concept which has found general acceptance then one must seek an alternative explanation so that ACTH presumably affecting a reaction common to all steroidogenic tissues acts only on adrenal cortical cells and luteotropic hormone possibly affecting the same reaction acts specifically on corpus luteum Such an alternative would postulate a specific affinity of tropic hormones for the target organs a notion akin to older pharmacological concepts and not in keeping with the more modern view that the specificity of hormone actions results from hormonal participation upon a specific tissue enzyme system When agents of differing chemical nature (compare ACTH to the luteotropic hormone) affect the same enzyme system one begins to wonder whether these specific tropic hormones directly act on the enzyme system at all

Consider as an alternative a situation where the cholesterol substrate and the degradation enzyme are arranged in the cell in a definite pattern as spatially related particles but too far apart to react chemically Let us suppose that the action of the tropic hormones is to bring the substrate and enzyme together at which point the reaction is initiated Viewed in this manner tropic hormones would resemble ATP action on muscle where in the presence of the appropriate ions this agent produces an association of actin and myosin to form an actomyosin complex which is the contractile element of muscle (21)

Once the hypothetical cholesterol degradation reaction has occurred and pregneninolone is formed the characteristic enzymes for the specific steroidogenic organs whether present in mitochondria or in other cytoplasmic constituents would take over to produce the specific steroids put out by these organs If one assumes that ACTH and luteotropic hormone act in this fashion then the next question concerns the nature of the forces which hold systems of this sort apart whether actin myosin or the postulated cholesterol degradation enzyme In the last analysis this resolves itself to the problem of the overall charge of particles governed by the ionic balance of the medium When the balance of the attractive and repulsive forces are altered appropriately association takes place The tropic hormones act in a minute concentration (in the case of ACTH the order of magnitude is gamma per liter) and it is inconceivable that the tropic hormones possess enough charge to influence any mechanism such as I have described But if tropic hormones were absorbed on the cell surface in a specific way and thereby influenced the permeability of ions (potassium and sodium) it is apparent that such an effect would provoke a profound change in the entire ionic environment of the cell On the basis of this speculation

TABLE XVIII

Capacity of Bovine Adrenals to Produce 11 Oxysteroid

Steroid	Blood Concentration (mg/l)	F plus Corticosterone (mg/hour of perfus o)
Cholesterol?	*ca* 2500	*ca* 0
Cholesterol? plus ACTH	*ca* 2500	*ca* 1
Pregnenınolone	25	*ca* 10
Progesterone	70	*ca* 25
DCA or S	200	*ca* 100

Rep t d by perm o fro Hecl t O *et al* Nature a d b ogene of ad c il r tory pro lict *Re e it Progr Hormone Resea* 1 6 215 (1951)

fluence of ACTH upon pregnenınolone or progesterone conversion to 11 hydroxycorticosteroid However the suggestion which arises from this work is that ACTH probably participates in the reactions before pregnenınolone presumably in a step between cholesterol and pregnen inolone On this basis ACTH would control the degradation of the cholesterol side chain by some adrenal enzyme system The nature of such an enzyme system might be envisaged as involving stepwise re moval by 2 carbon degradations to a C 21 stage Alternatively a system capable of a one step cleavage of the carbon to carbon bond between C 20 and C 22 might be involved In any case the suggestion is that ACTH would be involved in a system responsible for cholesterol side chain degradation

I should like to point out at once that having placed ACTH action in corticosteroid biosynthesis at such a point certain complications im mediately arise for this statement says that the action of ACTH is not involved in those reactions (e g 11 or 21 hydroxylation) which lead to the steroids specific for the adrenal cortex but in a step leads to pregnenınolone a steroid not specific for adrenal cortex at all From the work of Samuels *et al* (19) wherein pregnenınolone was shown to be transformed to progesterone in a variety of tissues (but particular ly in corpus luteum) it is possible to speculate that the reaction of cho lesterol to pregnenınolone may well occur as a general reaction in all hormonal steroidogenic organs with the possible exception of the ovarian follicle If ACTH affects this reaction in the adrenal what is the situation in the corpus luteum where cholesterol is presumably also a precursor pregnenınolone a likely intermediary and where progeste rone is an end product of luteal metabolism? Would ACTH affect the cholesterol pregnenınolone reaction in corpus luteum and thus lead to increased production of progesterone from the luteal tissue? If this

have been shown in homogenates I have already indicated why we would not consider ACTH as involved in these steps

Long Dr Hechter it is very interesting speculation Let s have some more First of all I think you are taking a rather narrow view as to the action of ACTH on the adrenal I should like to point out that it does other things It makes the adrenal grow for one thing and removal of ACTH causes atrophy of the adrenal Now is it not possible that ACTH has nothing at all to do with steroidogenesis and that the function of ACTH is concerned with what for want of a better term we might call the support of the cells in which these reactions can take place?

One more question Has there been any evidence at all that in a homogenate ACTH influences any of these reactions? In other words as I understand it the effects of ACTH can only be demonstrated when you have intact living adrenal cortical cells

Hechter To answer your second question first I know of no evidence that ACTH has an effect on steroid biosynthesis in adrenal homogenates With respect to your remarks regarding the support of cells by ACTH I must agree with you that ACTH does make an adrenal gland grow However in an isolated *in vitro* system ACTH also makes adrenal tissue synthesize corticosteroid I can study cortiscosteroid synthesis *in vitro* and the influence of ACTH upon biochemical reactions in adrenal tissue As yet it is very difficult although I don t say it is impossible to make an adrenal gland grow *in vitro* under the influence of ACTH One is a very simple reaction perhaps involving ACTH participation in a single reaction Growth however is a complicated question I don t know how to begin to study ACTH action at a molecular level in a growth process Perhaps in the simpler system it may be possible to begin to elucidate ACTH action in basic fundamental terms

Long I am not challenging you to make a perfused organ of any kind grow But if you enlarge your concept you do get away from the difficulty which you have pointed out of three tropic hormones influencing a common chemical transformation The tropic hormone is specifically *concerned with cells that carry out certain types of reactions and* does not necessarily participate in the reactions leading to the formation of the hormone

Hechter I agree that is a possible manner for ACTH action

Bloch Don t you introduce an unnecessary difficulty by assuming pregneninolone is an intermediate in all cases? You could for instance postulate that one of the first steps is the 11 hydroxylation with cholesterol

Hechter In the case of the isolated perfused adrenal it would appear

steroidogenesis in an isolated adrenal does not begin to look very different in essence, from muscle contraction or nerve conduction In all of these events a common denominator permeability of the surface and ion interchange, becomes a mediator whereby different stimuli produce diverse end responses

Jensen Dr Hechter, I think you meant that as pure speculation

Hechter Yes it should be labeled as wild speculation

Jensen I think one can approach the mechanism of steroidogenesis in another way One can assume that ACTH only causes the release of cortical hormones and that the lowering of these hormones produces an acceleration of corticosteroid formation There is simply a shift toward increased hormone production The rate of reaction follows the law of mass action

Hechter Our basic scheme of corticosteroidogenesis is a mixture of one part experimental findings and five to ten parts deduction The point of ACTH action in this metabolic pathway was deduced from a consideration of reaction rates on the primary assumption that the original scheme of corticosteroid biosynthesis is correct From this point forward one assumption led to another so that the final view of ACTH action presented may be described as an assumption built on an assumption built on an assumption all resting on a basic substructure which is certainly neither firm nor solid The working hypothesis which I have presented may be severely criticized at many points at each of these alternative possibilities exist I think you have raised one of these alternatives In my view however our findings seem best explained on the basis that biosynthesis precedes release adrenal cortical tissue having only a limited capacity to store corticosteroid rather than that biosynthesis follows as a consequence of accelerated release If you question the basic scheme of steroidogenesis presented I shall admit that our evidence is incomplete and is far from being definitely established It happens to be the best evidence we know of therefore we use it I can t answer your question in any other way

Jensen I am frank to say that I don t think you have any evidence

Hechter Let s go back to the beginning

Long No we won t go back to the beginning if you don t mind

Loewi Did you not say that some of this synthesis and this release goes on also in homogenates? Who did this work with homogenates? If you have a homogenate then you cannot have a surface action in my opinion That is why homogenates are so very valuable because you can exclude the surface of the cells

Hechter One fundamental point in my thesis is that certain but not other reactions proceed in homogenates The reactions from progesterone and 17 hydroxyprogesterone to corticosterone and compound F

where such different functions as secretion and synthesis are accomplished by the same agent

I should like to touch upon another point You said first you were not quite sure whether the blood was needed only for the supply of oxygen or for something else in addition Perhaps you could decide this by making the following experiment separate in the blood you are using the red cells from the plasma treat the red cells with carbon monoxide put them back in the plasma and check what will result from perfusion with such a blood

Finally I should like to ask you whether you determined the cholesterol and also the cholesterol esters in the blood before and after it had been used for perfusion?

Hechter No

Loewi If you think that perhaps the cholesterol ester of the blood would be used in the adrenal don t you think you should make an estimation of the cholesterol in the blood which comes in and which goes out?

Hechter I think that would be a very useful experiment and the ones which you propose are well worth doing For one reason or another we have not been able to study these questions The question of the importance of the red cells for ACTH induced steroidogenesis is certainly an important point and merits further investigation which we hope to do during the next year

With respect to the support work I can only repeat what I ve said I do not know how to study work or support of adrenal cortical tissue by ACTH at either a molecular or cellular level However I can test ACTH action in a relatively simple set of biochemical reactions

Long You could possibly study whether under the influence of ACTH there is active nitrogen retention in your gland Work measurement would be a little difficult in terms of the adrenal What is the oxygen exchange across the gland? What is the rate of oxygen consumption in relation to the rate of secretion?

Pincus There is also another interesting and very direct approach which we plan to use and that is the effect of temperature All of this work has been done at body temperature We do not have any idea what would happen at lower temperature

Loewi Is there any possibility of increasing the synthesis however it goes by ATP?

Hechter Martha Vogt has reported that both ATP and creatine phosphate stimulated synthesis of corticosteroids in isolated perfused glands (22) In addition she has reported that high potassium in the perfusion medium increased corticosteroid release I might say that her experiments would fit in very nicely with a hypothesis of the sort that I have just described

that once the 11 hydroxyl position is introduced as with 11 hydroxy progesterone, corticosterone and 17 hydroxycorticosterone further chemical transformations by adrenal enzyme seem to be halted This rule may not hold for the postulated 11 hydroxycholesterol you mention But in that case our entire scheme of steroidogenesis begins to fall apart As I indicated previously it all depends on which point you challenge the assumptions made

Bloch It sems to me there are great pitfalls in concluding from the conversion of A to B that this reaction lies in the normal path I am sure that you can take a large number of compounds which will be converted to a biologically active material and are not normal intermediates There are hundreds of such examples in biochemistry And from the ability of a substrate to react you cannot conclude that it is a normal intermediate For this reason I think the assumption of pregneninolone as an intermediate is not necessary

Hechter We appreciate that pregneninolone may not necessarily be a normal intermediary in corticosteroid synthesis from cholesterol As a matter of fact our entire scheme of corticosteroidogenesis suffers from similar limitations since it illustrates only possible reactions which may occur based upon ability of substrates to react At no point can we say with certainty that the specific intermediaries we postulate are necessarily the normal intermediates We have repeatedly emphasized that this is a tentative scheme that further work particularly *in vivo* experimentation is necessary However before these studies little or nothing was known concerning the metabolic pathways involved in corticosteroid synthesis This scheme of steroidogenesis represents a beginning perhaps inaccurate in many details but a beginning We use our scheme not as a final picture but as a guide for future experimentation This represents its sole function If future work indicates that the hypothesis must be modified or even discarded I would not be disturbed In the process of critical testing of some hypothesis better understanding of metabolic routes will undoubtedly be achieved

Loewi I like the remark of Dr Jensen He says the only thing we definitely know is that under the influence of ACTH more of the hormones are secreted Couldn t it be that if there is a strong excretion going on this has to be compensated for and that what you call the support may be the consequence of this strong excretion which incites a stronger activity to replace what is gone in other words work hypertrophy? It might be that this interpretation is too simple In fact the only thing that we know so far of the action of ACTH is that it stimulates the secretion of the adrenal cortex My assumption that the synthesis might be secondary to the secretion and not due to a direct function of ACTH is based on the fact that I do not know of any case

ing to pick out one isolated process in the whole complex cellular mechanism which is perhaps very indirectly influenced by the tropic agent

Hechter I agree that the adrenal glands grow when you give ACTH I also agree that they produce steroids and that they do this almost instantaneously when ACTH is administered However the two events may or may not be related to one another in a direct way

Astwood Perhaps the most specific statement one could make is that the tropic hormone stimulates the cell

Hechter Yes

Ralli There could be no argument with that

Conn May I make a clinical comment? The facility with which the perfused adrenal and homogenates of adrenal cortex convert 17 hydroxydesoxycorticosterone to F brings up inasmuch as we now know the metabolic effects in man of compound F an interesting situation If one gives normal people tremendous doses of compound S there are *essentially no metabolic effects whatsoever* With 400 mg a day either parenterally or by mouth with good evidence of absorption as indicated by a fourfold rise in 17 ketosteroids when S is given orally there is no evidence of any compound F like activity metabolically I realize that there are lots of things that probably happen between the administration of compound S and its possible circulation through the adrenal cortex in man But I think it is a point that should be made

Pincus I think you should devise a means of getting compound S

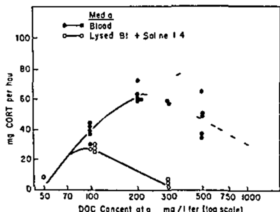


FIGURE 34 The effect of DCA concentration upon the rate of 11 hydroxylation in bovine adrenals Each dot or circle represents an individual experiment wherein the flow rate was approximately 1 liter per hour The dots represent experiments where DCA was perfused in whole blood the circles refer to DCA perfused with hemolyzed blood diluted with physiological saline 1 to 4 Reprinted by permission from Hechter O *et al* Nature and biogenesis of adrenal secretory product *Recent Progr Hormone Research* 6 215 (1951)

Nelson I want to comment briefly on an observation we have made in our work with the cannulated renal vein in the dogs We have observed that immediately following the intravenous administration of ACTH, there is an increase in the total blood flow from the gland and Dr Gassner has made a similar observation in the case of the cow *

Ralli How long is that maintained?

Nelson Over a period of about an hour

Hechter With the older ACTH preparations (possibly contaminated with posterior pituitary vasoconstrictors) corticosteroid biosynthesis in perfused glands was observed when the blood flow was reduced This would suggest that the action of ACTH in stimulating biosynthesis is independent of increased blood flow

Long But it would be fairly easy to measure oxygen consumption in those glands in your system before and after ACTH stimulation

Hechter It is fairly easy and it is very interesting I regret to say that we have not done that as yet

Ingle Dr Hechter isn t it true that the perfused adrenal begins to increase its rate of release of steroids within a few seconds after ACTH is introduced into the system?

Hechter ACTH action within thirty seconds is the shortest interval we have measured

Ingle The rapidity of that response makes it rather difficult to assume that growth is primary to an increased rate of secretion in that case

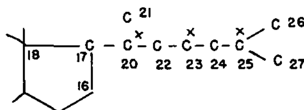
Hechter I am glad to hear you say that

Loewi A few years ago W O Fenn published the results of experiments performed on the submaxillary gland They demonstrated that during stimulation of the chorda tympani much potassium left the gland with the saliva and yet at the end of the stimulation the loss of potassium from the gland had been completely replaced from the blood It might be that the loss of some blood constituent could be replaced with the same speed

Astwood One could think of other instances where work hypertrophy induced by the work involved in resynthesis—the primary action of a hormone being on secretion—cannot be the whole story In the case of the thyroid gland synthesis of thyroid hormone can be completely blocked by an antithyroid drug and yet long after secretion is no longer possible the gland continues to grow under the influence of thyrotropin I would rather think that Dr Long s suggestion is the more appropriate one that the whole cell is influenced by these tropic hormones I am sure Dr Hechter only means to imply that he is try

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* Unpublished observations



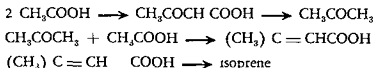
= ACETATE CH_3

x = ACETATE COOH

FIGURE 35 Reprinted by permission from Wuersch J Huang R L and Bloch K The origin of the isooctyl side chain of cholesterol *J Biol Chem* 195, *439 (1952)*

ued the chemical degradation of cholesterol which is formed in liver from labeled acetate (23) The distribution which is found in the cholesterol side chain in shown in Figure 35 We had previously identified the origin of the angular methyl groups (24) and found that of the eight carbon atoms in the side chain five are derived from the methyl group of acetic acid and three from the carboxyl group of acetic acid This by itself tells little about the nature of the intermediate reactions Acetate goes to the steroid structure From this point on everything is speculative

A year or two ago a paper was published by Bonner and Arreguin (25) who were interested in the biogenesis of natural rubber a polyisoprenoid compound They carried out *in vitro* experiments with isolated guayule leaves and found that acetate acetone and β dimethyl acrylate are very actively used in rubber formation and they postulated the following series of reactions



$$2\ CH_3COOH \longrightarrow CH_3COCH\ COOH \longrightarrow CH_3COCH_3$$

$$CH_3COCH_3 + CH_3COOH \longrightarrow (CH_3)\ C = CHCOOH$$

$$(CH_3)\ C = CH \quad COOH \longrightarrow \text{isoprene}$$

We have considered the relevance of this scheme to the biosynthesis of cholesterol (13) and we find that a mechanism utilizing acetate in this fashion would indeed give rise to the isotope distribution which we have observed From Figure 36A it can be seen that the distribution of acetate carbons in the side chain is in accord with the concept that isoprenoid units are intermediates in the conversion of acetate to cholesterol

into the adrenal arterial tree and then you will see Its dilution in the blood, and the other possible things that might happen to compound S might prevent its reaching the adrenal circulation in appreciable concentrations We have perfused compound S through livers for example and the rate of disappearance is certainly comparable to that which Dr Hechter mentions for desoxyticosterone and cortisone

Hechter Figure 34 I think will answer your question Dr Conn It illustrates the rate of corticosterone formed per hour of perfusion with varying concentrations of desoxycorticosterone (The blood flow was held constant at 1 liter per hour) It will be observed that the amount of corticosterone formed is a function of the concentration of desoxycorticosterone perfused When blood containing 100 mg per liter of desoxycorticosterone is directly introduced into the adrenal about 40 mg of corticosterone is formed per hour But please note the sharp reduction in rate when lower concentrations are perfused The point which Dr Pincus raised is essentially the crucial one How can you inject Reichstein s S or desoxycorticosterone in concentrations approaching 100 mg per liter directly into the adrenal enzymatic machinery? It would be only under circumstances such as these that one could expect significant amounts of compound F or corticosterone from substance S or desoxycorticosterone respectively

Conn Do you think it is possible to demonstrate evidence of conversion of S to F if one were to inject intravenously 400 mg of compound S? Would there be sufficient concentration in the adrenal artery to show anything?

Hechter The capacity of the liver to transform large amounts of corticosteroid rapidly was pointed out during Dr Nelson s presentation

Pincus Did you try this in a cirrhotic individual?

Conn We have

Hechter And it doesn t work does it?

Conn No

Hechter Try injecting Reichstein s S into the adrenal artery so that the liver and other metabolic organs would be bypassed

Long Perhaps Dr Bloch would utilize the remaining few minutes to say something about these acetylization reactions which he was invited to comment on and which certainly are a very important part of the whole story

Bloch * What I have to say is rather marginal to this discussion and therefore perhaps not as controversial At the first Conference I presented some evidence for the participation and utilization of C 2 units in cholesterol synthesis In the intervening two years we have contin

* This work is being supported by a grant from the Life Insurance Medical Research Fund

Perhaps you are all aware that many years ago organic chemists (e g Heilbron Robinson) suggested a biological relationship between squalene and the steroids Squalene is a hydrocarbon composed of six isoprene units It is a biochemical oddity in the sense that its main natural source is the liver oils from sharks Only recently MacKenna *et al* (26) have shown that squalene occurs in appreciable amounts in the unsaponifiable fraction of sebaceous secretions in human skin

One can at least on paper fold this squalene molecule in such a fashion that it can be superimposed on cholesterol We have made the further assumption that the isoprene subunits of squalene are synthesized by the mechanism of Bonner and Arreguin One then obtains a postulated isotope distribution in squalene as indicated in Figure 56B If these two structures are superimposed methyl and carboxyl carbon atoms of acetate appear in corresponding locations

The crucial experiment to test these speculations is of course to show a metabolic relationship between squalene and cholesterol Dr Langdon in our laboratory has tried to prepare squalene by organic synthesis The great difficulty is that one ends up with isomers which are biologically inert He has however recently succeeded in preparing squalene by biosynthesis in the rat If you analyze rat tissues you cannot detect squalene However if you feed normal squalene isolated from shark liver oils together with labeled acetate and then work up the unsaponifiable fraction you can recover labeled squalene which shows that squalene is synthesized in mammalian tissue but is apparently so active metabolically that it never accumulates normally to any appreciable degree

This is what we have done so far We hope to be able to make enough labeled squalene by biosynthesis to test the conversion to cholesterol Recently C 14 squalene prepared biosynthetically from labeled acetate has been fed to mice The cholesterol isolated subsequently from the tissues had a very high C 14 content demonstrating a utilization of squalene carbon for cholesterol synthesis There is no proof as yet that this conversion is direct but the formation of C 2 units as intermediates can be excluded

Hechter Have you tried isoprene incubated?

Bloch Isoprene boils at 34 C and is therefore difficult to administer to an animal

Hechter I was wondering if you could dissolve this gas in your media with your liver slices and if it would go to cholesterol?

Bloch We are testing various five carbon compounds which are easier to handle than isoprene

Ralli It is also interesting that cholesterol and carotene seem to bear some relation to one another in blood serum Where you find caro

A

o ACETATE CH_3

x ACETATE COOH

CHOLESTEROL $C_{27}H_{46}O$

A. DISTRIBUTION OF ACETATE CARBON FOUND IN CHOLESTEROL

B

SQUALENE $C_{30}H_{50}$

B POSTULATED DISTRIBUTION OF ACETATE CARBON IN SQUALENE

FIGURE 36 Reprinted by permission from Bloch K *Ciba Foundation Conference on Isotopes in Biochemistry* Wolstenholme G E W Editor Ciba Foundation 1951 (p 25)

12 Srere P A Chaikoff I L and Dauben W G *In vitro* synthesis of cholesterol from acetate by surviving adrenal cortical tissue *J Biol Chem* **176** *829 (1948)*

13 Bloch K The use of isotopes in hormone problems the biological synthesis of cholesterol *Recent Progr Hormone Research* **6** *111 (1951)*

14 Hayano M Dorfman R I and Yamada E Y Conversion of desoxycorticosterone to glycogenic material by adrenal homogenates *J Biol Chem* **193** *175 (1951)*

15 Savard K Green A A and Lewis L A Oxidation of 11 desoxycorticosteroids with adrenal tissue homogenates *Endocrinology* **47** *418 (1950)*

16 McGinty D A *et al* Biosynthesis of 17 hydroxycorticosterone from 11 desoxy 17 hydroxycorticosterone *Science* **112** *506 (1950)*

17 Sweat M L Enzymatic synthesis of 17 hydroxycorticosterone *J Am Chem Soc* **75** *4056 (1951)*

18 Hayano M and Dorfman R I The enzymatic conversion of progesterone 17 hydroxyprogesterone and 21 desoxycortisone *Arch Biochem & Biophys* (In press)

19 Samuels L T *et al* An enzyme in endocrine tissues which oxidizes Δ^5 3 hydroxy steroids to $\alpha\beta$ unsaturated ketones *Science* **113** *490 (1951)*

20 Green D E Enzymes and trace substances *Advances Enzymol* **1** *177 (1941)*

21 Szent Gyorgyi A *Chemistry of Muscular Contraction* New York Academic Press 2nd edition 1951 (p 29)

22 Vogt M Cortical secretion of the isolated perfused adrenal *J Physiol* **113** *129 (1951)*

23 Wuersch J Huang R L and Bloch K The origin of the iso octyl side chain of cholesterol *J Biol Chem* **195** *439 (1952)*

24 Little H N and Bloch K Studies on the utilization of acetic acid for the biological synthesis of cholesterol *J Biol Chem* **183** *33 (1950)*

25 Bonner J and Arreguin B The biochemistry of rubber formation in the guayule I Rubber formation in seedlings *Arch Biochem* **21** *109 (1949)*

26 MacKenna R M B Wheatley V R and Wormall A The composition of the surface skin fat (sebum) from the human forearm *J Invest Dermat* **15** *33 (1951)*

tenemia you invariably find an increase in the serum level of cholesterol

Bloch Mechanisms for the biosynthesis of terpenes vitamin A steroids etc using a common five carbon precursor, have been favorite topics of speculation for many years The tools are now available to test this hypothesis The postulated squalene cholesterol relation is attractive to us because the formation of a long chain compound of this nature permits the introduction of branchings which become angular methyl groups in the steroids at an early stage of the synthetic process It is more difficult to visualize the attachment of angular methyl groups to a completed ring system

I should mention a very serious flaw in the scheme which I have presented It does not make allowance for the synthesis of the various plant steroids which have additional carbon atoms in the aliphatic side chain

REFERENCES

1 HECHTER O *et al* Nature and biogenesis of adrenal secretory product *Recent Progr Hormone Research* 6 *215 (1951)*

2 ZAFFARONI A BURTON R B and KEUTMANN E H Adrenal cortical hormones analysis by paper partition chromatography and occurrence in urine of normal persons *Science* 111, 6 *(1950)*

3 NELSON D H SAMUELS L T and REICH H The cortical steroid in mammalian blood after ACTH stimulation *Proc Sec ond Clin ACTH Conf Vol 1* Philadelphia The Blakiston Co 1951 (p 49)

4 BUSH I E Paper chromatographic study of secretion of adrenal cortex in various mammalian species *J Physiol* 115 *12P (1951)*

5 PINCUS G HECHTER O and ZAFFARONI A Effect of ACTH upon steroidogenesis by isolated perfused adrenal gland *Proc Second Clin ACTH Conf Vol 1* Philadelphia The Blakiston Co 1951 (p 40)

6 LONG C N H Relation of cholesterol and ascorbic acid to secretion of adrenal cortex *Recent Progr Hormone Research* 1 *99 (1947)*

7 OESTERLING M J and LONG C N H Adrenal cholesterol in scorbutic guinea pig *Science* 113, *241 (1951)*

8 HAINES W J *et al* Biosynthesis of 17 a hydroxycorticosterone from acetate *Arch Biochem & Biophys 32, 218 (1951)*

9 MUNSON P L BARRY A G JR and KOCH F C A simplified hypophysectomized rat adrenal ascorbic acid bioassay method for ACTH specificity and application to preparative problems *J Clin Endocrinol* 8 *586 (1948)*

10 SAYERS M A SAYERS G and WOODBURY L A Assay of adrenocorticotropic hormone by adrenal ascorbic acid depletion method *Endocrinology* 42, *379 (1948)*

11 PAYNE R W RABEN M S and ASTWOOD E B Extraction and purification of corticotropin *J Biol Chem* 187 *719 (1950)*

12 SRERE P A CHAIKOFF I L and DAUBEN W G *In vitro* synthesis of cholesterol from acetate by surviving adrenal cortical tissue *J Biol Chem* 176 *829 (1948)*
13 BLOCH K The use of isotopes in hormone problems the biological synthesis of cholesterol *Recent Progr Hormone Research* 6, *111 (1951)*
14 HAYANO M DORFMAN R I and YAMADA E Y *Conversion* of desoxycorticosterone to glycogenic material by adrenal homogenates *J Biol Chem* 193, *175 (1951)*
15 SAVARD K GREEN A A and LEWIS L A Oxidation of 11 desoxycorticosteroids with adrenal tissue homogenates *Endocrinology* 47, *418 (1950)*
16 McGINTY D A *et al* Biosynthesis of 17 hydroxycorticosterone from 11 desoxy 17 hydroxycorticosterone *Science* 112 *506 (1950)*
17 SWEAT M L Enzymatic synthesis of 17 hydroxycorticosterone *J Am Chem Soc* 75, *4056 (1951)*
18 HAYANO M and DORFMAN R I The enzymatic conversion of progesterone 17 hydroxyprogesterone and 21 desoxycortisone *Arch Biochem & Biophys* (In press)
19 SAMUELS L T *et al* An enzyme in endocrine tissues which oxidizes Δ^5 3 hydroxy steroids to $\alpha\beta$ unsaturated ketones *Science* 113 *490 (1951)*
20 GREEN D E Enzymes and trace substances *Advances Enzymol* 1, *177 (1941)*
21 SZENT GYORGYI A *Chemistry of Muscular Contraction* New York Academic Press 2nd edition 1951 (p 29)
22 VOGT M Cortical secretion of the isolated perfused adrenal *J Physiol* 113 *129 (1951)*
23 WUERSCH J HUANG R L and BLOCH K The origin of the iso octyl side chain of cholesterol *J Biol Chem* 195 *439 (1952)*
24 LITTLE H N and BLOCH K Studies on the utilization of acetic acid for the biological synthesis of cholesterol *J Biol Chem* 183 *33 (1950)*
25 BONNER J and ARREGUIN B The biochemistry of rubber formation in the guayule I Rubber formation in seedlings *Arch Biochem* 21 *109 (1949)*
26 MACKENNA R M B WHEATLEY V R and WORMALL A The composition of the surface skin fat (sebum) from the human forearm *J Invest Dermat* 15, *33 (1951)*

CLINICAL USE OF ADRENAL CORTICAL HORMONES AND ACTH

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CLINICAL DATA are quite different from the type of material so far presented but I believe that a partial review of the clinical use of cortisone and ACTH principally in the institution that I come from may be of interest to you There are three aspects to the present use of cortisone and ACTH by my associates and myself First a systematic effort is under way to learn how to use the hormones effectively in treat ment while avoiding insofar as possible their undesirable effects Secondly we are gradually acquiring as is everyone else a further evaluation of the actual worth of cortisone and ACTH in the therapy of various diseases Lastly some physiologic observations in human sub jects are being made

It has been my belief for some time and I think this opinion is shared by many others that states of adrenal cortical insufficiency and pos sibly also of congenital adrenal hyperplasia are really the only condi tions in which the use of these hormones can properly be designated as physiologic In conditions such as rheumatoid arthritis in which depression of adrenal cortical function does not seem to play a causa tive role it would appear that the therapeutic activity of cortisone and ACTH is better described as pharmacologic In a recent paper entitled *The Integrative Action of the Endocrine System* Means (1) forcibly distinguished between the use of the hormones as replacement therapy and their use in the pharmacologic sense

It may appear paradoxical to state that the action of a substance which occurs naturally in the body is physiologic under one set of cir cumstances and pharmacologic under another An important point of distinction is the amount of the hormones which must be administered in order to achieve a therapeutic effect under the two different circum stances In Addison s disease for example the dose which is necessary for maintenance is presumably roughly equal to the normal output of the adrenal cortices In rheumatoid arthritis on the other hand the amount which must usually be employed is several fold larger than in Addison s disease and probably in excess of what is normally produced by the adrenal cortices

Although there is as yet no proof of the foregoing evidence is accumulating which points in that direction Perhaps work such as Dr Nelson is doing that is measuring blood levels of the steroids under varying conditions will shed further light on this general question In this connection the values for urinary corticosteroids (formaldehydogenic steroids) which are obtained before and after enzymatic hydrolysis with beta glucuronidase are of interest In Dr H L Mason s laboratory the maximal normal value for free corticosteroids is 1 2 mg in twenty four hours Following enzymatic hydrolysis however a group of normal persons were found to excrete from 10 to 30 mg with an average of 15 mg of total corticosteroids in twenty four hours After administration of cortisone or compound F in doses of 100 or 200 mg daily the values for free corticosteroids in four cases were 91 0 20 7 25 6 and 46 2 mg in twenty four hours Thus although the figures cited for total corticosteroid excretion by normal subjects suggest that the adrenal cortex produces more steroidal material than was formerly suspected the values for free and total corticosteroids after administration of therapeutic doses of cortisone or compound F indicate that these doses exceed the normal amount of secretion Further data of this type should be obtained

Another indirect bit of evidence concerning the rate of corticosteroid secretion by the human adrenal cortex is the amount of cortisone which is necessary to restore diabetes to full severity in patients with coexisting Addison s disease and diabetes We have had an opportunity to study three such patients and have found that doses of 25 mg or less of cortisone each day completely restore the daily insulin requirement to what it was prior to the development of adrenal insufficiency

On the basis of this and other evidence it would appear that therapeutic doses of 100 mg of cortisone daily are in excess of the normal daily output of this type of steroid by the adrenal cortices Therefore such doses are not replacement doses and their effects would seem to be pharmacologic rather than physiologic

I am aware that there are certain lines of evidence which indicate a reduced function of the adrenal cortices in rheumatoid arthritis My personal appraisal of this is that the reduced function might as well be a result as a cause of the disease I know of no convincing evidence that an insufficiency of adrenal hormones is a factor in the pathogenesis of any of the inflammatory diseases of connective tissue

Ralli It is important to stress that probably adrenal insufficiency is not necessarily an important factor in diseases in which cortisone is effective

Astwood Another theory a variant of the one that Dr Sprague mentioned might be worthy of thought Perhaps in human beings

susceptible to these diseases, the adrenal does not respond adequately to the stress of the disease and consequently the disease is somehow permitted to occur By the time the disease has developed measurements would not show a difference from the normal

Sprague We have measured corticosteroid excretion of patients during flares of rheumatoid arthritis In some instances we have found an increase in corticosteroid excretion presumably in response to the stress of the disease

Pincus Has Dr Mason done any qualitative analyses of these urinary products? What you have said so far does not exclude the possibility of a steroid dysgenesis rather than a steroid insufficiency Are there any data which might contribute to that point?

Sprague Dr Mason has not carried out such isolation work Some of Dr Dobriner s data on urinary steroids with which you are familiar might have some bearing on the question Of course this whole problem is in a stage where one can conjure up almost any kind of a theory to explain what is wrong in rheumatoid arthritis

Conn Dr Sprague most patients who are given sufficient amounts of cortisone to affect the disease show evidence of temporary adrenal depression upon removal of the cortisone Would that not be suggestive of pharmacological rather than physiological doses?

Sprague It is consistent with the idea that we are using doses in excess of the physiologic amounts

Hechter Is there evidence of adrenal atrophy with small doses of cortisone administered orally?

Sprague We do not have any evidence on that

Hechter Do you Dr Conn?

Conn It depends on what one calls small doses

Hechter Doses of 25 mg orally per day

Conn There are very few patients if any who can be maintained on 25 mg daily other than patients with Addison s disease or hypopituitarism

Hechter What is the smallest dose which can be utilized to maintain a patient?

Thorn We have evidence of a temporary decrease in 17 ketosteroid output in a female patient who was maintained on 25 mg of cortisone per day This is not a patient with rheumatoid arthritis and it is true that she appeared to escape from this dosage after a period of several weeks We thought however that the initial observations were consistent with the findings of Dr L Wilkins (2) who certainly has observed an inhibition of adrenal cortical activity with low levels of cortisone administration

Sprague Dr Wilkins has striking evidence of suppression of adrenal

function by administration of relatively small doses of cortisone at least under the peculiar circumstances of congenital adrenal hyperplasia However I do not think this answers the question that Dr Hechter had in mind

Sayers May I ask if any studies on the metabolic pattern of patients with collagen diseases have been conducted and if so whether there is any indication that there is some defect in electrolyte or carbohydrate metabolism which could be assigned to deficiency of adrenal cortical function?

Sprague I know of no studies pointing in that direction There have been numerous metabolic studies of such patients incident to the use of cortisone and ACTH but I have not seen evidence which indicates any metabolic abnormality that could be attributed to deficient adrenal cortical function

Selye I think one should not dismiss too lightly the possibility of a hypofunction of the adrenal cortex in rheumatic diseases merely on the basis of evidence showing that the production or excretion of corticoids remains normal The theory according to which adrenal function is a causative factor in rheumatoid arthritis does not postulate that there is a decrease in *absolute* corticoid levels The question is whether these patients do not produce an insufficient amount of corticoids for the condition of disease in which they are? If I may use an analogy a man running fast will unfailingly develop circulatory disturbance if his heart beat continues to remain perfectly normal during strenuous exercise

In the problem which faces us the question is whether the production of corticoids is sufficient for the requirements induced by whatever the causative agent of rheumatoid arthritis might be The fact that non specific therapy is partially effective and at the same time causes evidence of increased glucocorticoid production would rather suggest to me that an auto pharmacologic defense is involved in this disease in that the adrenals do not produce the high excess of corticoids which the causative pathogen necessitates

Some recent experiments bear upon the question under consideration In rats which were heavily overdosed with desoxycorticosterone for a period of two weeks the blood pressure and renal structure were found to be essentially normal a few days after discontinuation of this treatment Yet all these animals eventually developed marked hypertension nephrosclerosis and periarteritis nodosa with evidence of widespread collagen disease long after the hormone overdosage was discontinued Apparently we had produced a change with this temporary hormone overdosage which set up a trigger mechanism eventually leading to the syndrome of hypertension and collagen disease I mention this because it illustrates that a condition of stress temporarily causing deranged or

excessive adrenal corticoid production may eventually become the cause of disease at a time when an excess of corticoid could no longer be demonstrable by urine assays

Therefore, I should like to ask Dr Sprague whether in the light of these observations, he does not feel the necessity of reconsidering his views according to which the adrenal cortex would not participate in the pathogenesis of rheumatoid arthritis In particular should not one admit the possibility that *(a)* there is not enough glucocorticoid material produced in rheumatoid arthritis to meet the stress situation in which the patients finds himself and *(b)* although the corticoids play an important part in the pathogenesis of these diseases absolute excretion values in the urine may remain within normal limits? Personally I should think it extremely improbable that a disease which has been reproduced in animals by excess mineralocorticoids and is so beneficially influenced both in animals and in man by glucocorticoid therapy should evolve without any participation of endogenous corticoids and that the beneficial action of the latter in patients is merely due to a pharmacologic action which is no more related to the evolution of this malady than aspirin would be involved in the pathogenesis of the headache which it alleviates

Ralli Didn t you have to condition your animals by giving them larger amounts of salt before you could produce this DCA effect?

Selye That is necessary only to get it with comparatively small doses *You cannot produce any of these collagen disease changes in animals* on sodium free diets but you can produce them on diets containing normal amounts of sodium as long as you correspondingly raise the dose of hormone (3 4) Dr Sidney Friedman of Vancouver recently confirmed this with various rat strains (5) Such sensitizing procedures as excess sodium or unilateral nephrectomy are facilitating factors only in the sense that they make it easier to obtain the experimental disease early and with comparatively small doses

Sprague In general I would agree that the possibility of some role of the adrenal cortex in the pathogenesis of these diseases should not be discarded particularly with the relatively meager evidence that we now have at hand However there is little support for the idea that the so called collagen diseases are due to an absolute deficiency of adrenal hormones Dr Thorn and others have pointed out that we should see a higher incidence of such diseases in patients with Addison s disease if this were the case It is difficult for me to accept the theory that these diseases are attributable to a deficiency of cortisone like steroids because the patients do not show other convincing evidence of such a deficiency One can counter by saying that the tissues which are most strikingly affected by the disease (for example the synovia in rheuma

toid arthritis) have a higher requirement for such steroids than other tissues of the body In my opinion this does not help to clarify the problem for the reason why certain tissues have a supernormal requirement for these steroids remains unexplained

I was impressed by the sequence of events in the case of Drs Hench Kendall Slocumb and Polley s first patient with rheumatoid arthritis to receive cortisone Starting in September 1948 she received cortisone daily for approximately five months in an average daily dose of 90 mg During that period many of the signs and symptoms of cortisone excess including moderately severe mental depression gradually developed in spite of the fact that the doses of cortisone were inadequate to control her arthritis completely In other words even though the doses of cortisone did not suppress her arthritis other signs of hormonal excess still developed This observation has now been repeated many times To me it indicates that when we administer cortisone to a patient with rheumatoid arthritis we are not simply supplying something which is lacking

Hechter I should like to raise a point concerning this term collagen diseases Is there actually any disturbance of the collagen fibres in any of the disease processes referred to as collagen diseases?

Bauer We are without evidence that these diseases are primarily disorders of collagen or that the frequently observed histologic feature fibrinoid is collagenous in origin Thus the term collagen diseases as currently used by many is misleading True collagen may be involved but so too are other connective tissue components and mesenchymal structures Lacking evidence that these seemingly related diseases of unknown etiology are due to disturbances of collagen there is an increasing tendency to use a less definitive nomenclature hence the introduction of such terms as connective tissue diseases and mesenchymal tissue diseases Such terms are less misleading in that they merely indicate the structures involved yet in no way imply the exact site of the initial lesion or its cause

Sprague I should like to discuss briefly the use of cortisone as replacement therapy in conditions of adrenal cortical insufficiency including Addison s disease adrenal insufficiency secondary to pituitary insufficiency and Cushing s syndrome immediately after surgical removal of hyperfunctioning lesions of the adrenal cortex

We have employed cortisone in the treatment of approximately twenty five patients with Addison s disease I should like to present the results of metabolic studies of two of these patients which seem to indicate that cortisone in these cases at least did not have sufficient electrolyte activity to maintain electrolyte equilibrium when the patients ingested a moderate amount of sodium chloride

The first patient was a woman aged forty two with Addison s disease of twelve years duration (Figure 37) After withdrawal of DCA *excretion of sodium and chloride* continued at an augmented rate throughout fifteen days of administration of cortisone acetate alone (periods 3 4 and 5) The dose was 100 mg on the first day followed by 50 mg daily During this time the hematocrit reading (cell volume per cent) rose progressively the plasma sodium and chloride decreased and clinical evidences of dehydration became apparent In the mean time however the patient felt quite well It was not until administration of DCA was reinstituted (period 6) in addition to cortisone, that salt was again retained normal concentration of plasma sodium and chloride were restored and the patient became normally hydrated Subsequently withdrawal of cortisone while DCA was continued (period 9) was not followed by any pronounced change in the excretion of sodium and chloride The sequence of events following withdrawal of all treatment for four days was studied (period 12) The changes in the plasma electrolytes and in electrolyte excretion were qualitatively similar to those previously observed on substitution of cortisone for DCA but they were of greater magnitude and less well tolerated from a clinical standpoint

Similar studies were carried out in the case of a seventeen year old boy with Addison s disease with the exception that administration of DCA in a dose of 3 mg daily was continued during the first six days (period 3) of administration of cortisone (Figure 38) Subsequently for eighteen days cortisone alone was administered in a dose of 50 mg daily (periods 4 5 and 6) During most of the eighteen days and *particularly on the first two days the excretion of sodium and chloride* was relatively large the values for plasma sodium and chloride decreased the hematocrit readings rose and dehydration became evident However in the last six days of treatment with cortisone alone (period 6) possibly because of accumulation of the salt retaining effects of the hormone urinary excretion of sodium and chloride was once more reduced to values comparable to those observed previously during treatment with DCA alone When administration of DCA in addition to cortisone was resumed (period 7) excretion of sodium and chloride decreased promptly and markedly

Bauer Was this with parenteral cortisone?

Sprague Yes the cortisone was administered intramuscularly in these studies

We have carried out a similar study on a third patient with Addison s disease with essentially *similar results It is of interest in* this case that 50 mg of cortisone acetate daily was not capable of maintaining the patient in electrolyte equilibrium even though the intake of sodium

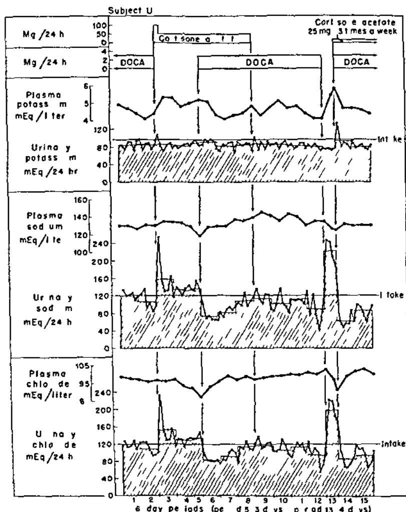


FIGURE 37 Effects of cortisone acetate alone and in association with desoxycorticosterone acetate on plasma and urinary electrolytes in a woman with Addison's disease Reprinted by permission from Sprague R G Mason H L and Power M H Physiologic effect of cortisone and ACTH in man *Recent Progr Hormone Research* *6 315 (1951)*

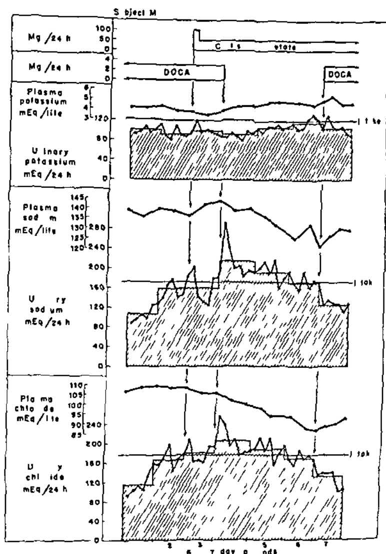


FIGURE 38 Effects of cortisone acetate alone and in association with desoxycorticosterone acetate on plasma and urinary electrolytes in a boy with Addison's disease Reprinted by permission from Sprague R G Mason H L and Power M H Physiologic effect of cortisone and ACTH in man *Recent Progr Hormone Research* 6 315 (1951)

and chloride was as high as 520 mEq a day While it is quite possible that cortisone might suffice as treatment in some cases of Addison s disease if used in conjunction with a high intake of sodium chloride we have made a practice on the basis of these studies of using a com bination of cortisone and DCA By using such a combination it has been a relatively simple matter to maintain patients with Addison s disease in electrolyte equilibrium with normal concentrations of plasma sodium chloride and potassium

Probably the most remarkable effect of cortisone which we have observed in our patients with Addison s disease has been what Dr Ingle has termed the vim and vigor effect Almost without exception these patients have felt stronger their appetites have improved they have tended to gain weight and they have in general been able to carry on more normal activity than they did before the use of cortisone Thus far there has been no change in any of the laboratory studies which we have made of these patients that seem adequate to explain the so called vim and vigor effect of cortisone Lymphocytosis which was com monly present prior to treatment frequently persisted after the institu tion of cortisone therapy Concentrations of plasma electrolytes which usually were maintained at normal levels during treatment with DCA remained normal after the institution of cortisone therapy Basal metab olic rates were not significantly changed There has frequently been a slight rise in the level of the fasting blood sugar but it is not of suffi cient magnitude to be impressive It can readily be demonstrated that cortisone enables patients with Addison s disease to withstand prolonged periods of fasting without hypoglycemia developing (Figure 39) How ever in our experience in most patients with Addison s disease sig nificant hypoglycemia does not develop with just an overnight fast It therefore seems difficult to explain the favorable clinical effects of cor tisone on the basis of improvement in blood sugar regulation Never theless its favorable effects may be related to its carbohydrate activity in some more subtle manner which has not yet been studied in patients

I have talked to Dr Ingle at various times about the so-called vim and vigor effect and I wonder if he might have any comments about it that would be pertinent here

Ingle I can only acknowledge that we do not know very much about the basis of the effect of the adrenal hormones upon vim and vigor We measure the potency of adrenal steroids and extracts by our muscle work test which we regard as a criterion of vigor and in general these results correlate very closely with the results of bioassays by the glyco gen deposition tests but there are some clear-cut dissociations In addi tion if one administers glucose to the adrenalectomized working animal and thereby prevents depletion of carbohydrate stores the animal re

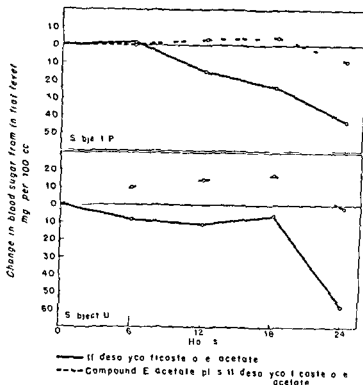


FIGURE 35 Improved ability of two patients with Addison's disease to maintain the level of blood sugar during fasting when treated with cortisone acetate (compound E acetate) Reprinted by permission from Sprague R G Mason H L and Power M H Studies of the effects of adrenal cortical hormones on carbohydrate metabolism in human subjects *Proc Am Diabetes A (1949) 9 149 (1950)*

mains incapable of sustaining work unless the hormones of the adrenal cortex are also administered The effect of these hormones upon vigor must have an important basis other than making carbohydrate available to the organism If there is some defect in carbohydrate utilization in the adrenally insufficient animal it has not been identified carbohydrate disappears rapidly enough in the adrenally insufficient animal

White I don t know very much about the so called vigor center but in the case of vim are we far enough along in our thinking with respect to the opposite effects of these two compounds on brain excitability to relate the matter of better feeling and greater stability that one finds with cortisone versus DCA and versus mixtures of the two to studies of brain excitability in rats? Is this work beginning to be a basis for interpreting the EEG changes?

Thorn I think that is the most important correlation namely that

the desoxycorticosterone treated patient can achieve a very satisfactory daily work output What Dr Sprague is speaking of is as much an attitude that is whether the patient is interested in working or carrying out a useful occupation We have always felt that the persistence of an abnormal electroencephalographic tracing in patients with Addison s disease treated with desoxycorticosterone constitutes one of the real indications for the use of cortisone regardless of how well physically the patient appears to be doing The striking effects in general in the patients given cortisone and the restoration of the electroencephalogram toward normal suggest that there has been a marked disturbance in the central nervous system

Pincus Have you done any testing of psychological functions in these patients for example of the latent periods reaction times and things of that sort?

Thorn Yes

Pincus Is there any differentiation between the DCA treated and others?

Thorn I can t answer that

Long I should like to ask about the blood sugar level Isn t there a progressive fall during the period when the electroencephalograms were taken?

Thorn Glucose never restored the electroencephalogram to normal

Long There is a striking difference of the order of 30 mg per cent in the blood sugar level between the cortisone treated and nontreated subject It is not insignificant if the initial blood glucose level is around 90 mg per cent

Thorn As Dr Sprague has pointed out the electroencephalographic change is a more or less permanent one in these patients It is not produced by a twenty four hour fast Abnormalities in the electroencephalogram are present before lunch as well as after lunch

Long But in six hours in sub U you have already a 2 mg per cent spread

Ralli Wouldn t it be true that the concentration of glucose in the circulating blood might still be elevated while the concentration available to the brain cells was slowly decreasing? There must be some explanation for the feeling of well being it isn t as if you just wafted a wand over them If not the blood sugar levels what might conceivably contribute to the cellular metabolism in the brain which alters their feeling toward life in general?

Pincus Studies of the effect of glucose on the EEG in fasting and hypoglycemia show that you have to go to rather low levels to get the abnormal slow waves which are typical of the Addisonian

Long There is a little more behind this than the blood glucose level

What is sustaining the blood glucose in the one case as against the other? There are other processes involved here presumably connected with gluconeogenesis

Pincus If you are talking about something behind all this yes but the direct relationship of glucose to the LEG is not the answer

Conn It should be remarked in this connection that compound B given to the Addisonian also confers the vim and vigor aspect also prevents the fasting hypoglycemia and tends to return the electro encephalogram toward normal That does not indicate anything other than that there are definite effects on organic metabolism of compound B However the effects of B upon organic metabolism are considerably less intense than those produced by cortisone

Ralli Have you had the experience of surgical procedures with these Addisonians such as I saw in one patient with an acute appendix a patient treated with cortisone? Is there any evidence that the ability to heal wounds is interefered with?

Sprague Our experience is limited to two major surgical procedures a cholecystectomy and a radical breast amputation and several minor ones on patients with Addison s disease treated with cortisone There was no difficulty with wound healing

I would be reluctant to accept the view that the well being of patients with Addison s disease during treatment with cortisone is all cerebral in origin Dr Ingle s demonstration that anesthetized adrenalectomized rats are able to perform more muscular work during administration of cortisone suggests that the hormone has a peripheral effect Dr E H Lambert has been carrying out some studies of muscle function in patients with Addison s disease in which he measures the strength of single muscle twitches following stimulation of a peripheral nerve There is evidence from these studies that cortisone improves neuro muscular function in these patients Nevertheless there is no reasonable doubt that the cerebral effects of cortisone are important

Ingle I should like to know if Dr Thorn would really insist that the Addisonian treated with 11 desoxycorticosterone acetate is normal in respect to ability to work

Thorn I did not say or mean to imply that the Addisonian patient treated with 11 desoxycorticosterone acetate is normal in respect to all of his body functions I merely stated that the patient receiving this treatment has the capacity to carry in most instances a reasonable day s work of muscular exercise It is important to recall that in Dr Ingle s test in order to fatigue the adrenalectomized animal within a reasonable length of time so that comparative studies could be made with normal animals it was necessary to nephrectomize them This gives us some idea that the normal support of electrolytes permits many patients

with Addison s disease to carry on reasonably well in terms of muscle function We all agree that the further addition of cortisone is a tremendous additional benefit particularly under stress

Ingle The majority of them can renew their usual activities but if they were pushed to the limit of their ability to do muscle work wouldn t you expect that they like the rat would do very poorly on 11 desoxycorticosterone acetate?

Thorn The only point I should like to reiterate is that cortisone not only facilitates the capacity to carry out muscular work but probably also helps provide the nervous energy necessary for prolonged effort We are all aware in clinical medicine that the greatest disability arises in those individuals with an adequate skeletal system but with disturbed psychological function who are unable to carry out an ordinary day s activities although it is obvious that their physical capacity to do so is really unlimited

Ingle As a relative point Dr C P Richter* has studied the effect of cortisone upon the voluntary activity of the rat When a rat is castrated its voluntary activity almost disappears it just sits When such an animal is treated with cortisone it becomes active again which is not a true measure of whether he has euphoria or not but it suggests that he might

White What happens to the activity of the castrated rat given desoxycorticosterone? Does he just sit?

Ingle DCA has a relatively small transitory effect on running activity

Long Does the cortisone treated rat eat more at the time he becomes more active?

Ingle I do not know

Sprague I should like to comment further on the question of dosage of cortisone in the treatment of Addison s disease The daily dose of virtually all patients whom we have treated has been between 10 and 25 mg usually given orally in divided doses We have the impression that some patients with Addison s disease are remarkably susceptible to the cerebral effects of cortisone Some patients who daily receive 25 mg or less will show so much evidence of stimulation insomnia euphoria and so forth that it becomes necessary to reduce the dose Much larger doses are usually necessary to produce similar effects in patients with intact adrenals On the other hand there is some evidence that patients with Addison s disease may eventually adapt themselves to doses of cortisone which initially produced excessive cerebral stimulation

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Ric [illegible] C [illegible] sun [illegible]

Reference has already been made to the ability of small doses of cortisone to restore the full severity of diabetes in patients having co existing Addison s disease and diabetes At this time I should like to show data obtained from experiments in the study of three such patients All of the steroids studied were *administered* intramuscularly

The first patient was a woman in whom both the Addison s disease and the diabetes were severe With the development of adrenal insufficiency the diabetes had been markedly ameliorated and a pronounced sensitivity to insulin had developed During periods of treatment with

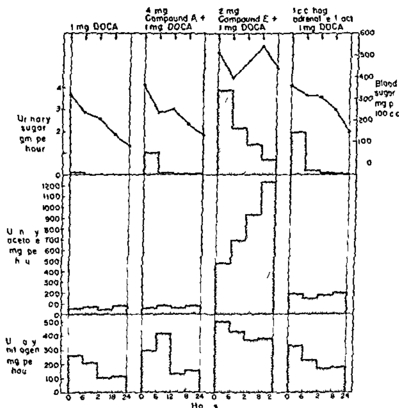


FIGURE 10 Changes in blood sugar and urinary sugar ketone bodies (measured as acetone) and *nitrogen* in a women with coexisting *Addison s* disease and diabetes *mellitus* The data were obtained during *periods of fa ting* after withdrawal of insulin while the steroids indicated at the top of the chart were being *administered* Reprinted by permission from Sprague R G Mason H L and *Power* M H Studies *of the effects* of adrenal cortical hormones on carbohydrate metabolism in human subjects *Proc Am Diabetes A (1949) 9 149 (1950)*

DCA alone insulin requirements were low and there was little or no glycosuria When insulin was withdrawn and she fasted during treatment with DCA alone or in combination with 11 dehydrocorticosterone (compound A) or hog adrenal extract in the doses shown in Figure 40 the sugar in the blood and urine decreased and there was only slight ketonuria By contrast during treatment with cortisone (com

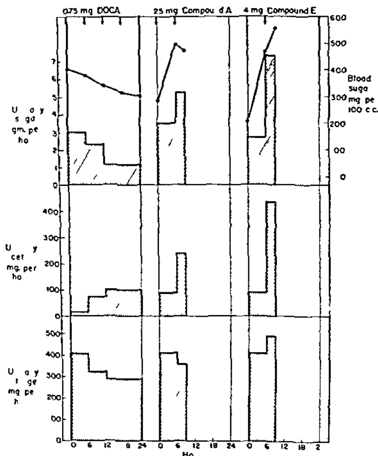


FIGURE 41 Changes in blood sugar and urinary sugar ketone bodies (measured as acetone) and nitrogen in a woman with coexisting Addison s disease and diabetes mellitus The studies were carried out in the same manner as those presented in Figure 40 Reprinted by permission from Sprague R G Mason H L and Power M H Studies of the effects of adrenal cortical hormones on carbohydrate metabolism in human subjects *Proc Am Diabetes A (1949) 9 149 (1950)*

pound F) the blood sugar was maintained at a high level glycosuria was more marked ketonuria was pronounced and the excretion of nitrogen was significantly greater than in the other experiments

The second patient was a woman who had severe diabetes which had been only slightly ameliorated by the development of adrenal insufficiency When she fasted after withdrawal of insulin during treatment with DCA the level of the blood sugar and the amount of sugar in the urine decreased (Figure 41) Ketonuria persisted but was not pronounced When she fasted during treatment with 11 dehydrocorticosterone (compound A) or cortisone (compound E) on the other hand there was a rapid rise in the blood sugar and intense glycosuria and severe ketosis developed The effects were most pronounced when cortisone was given although only two doses of 4 mg each were ad

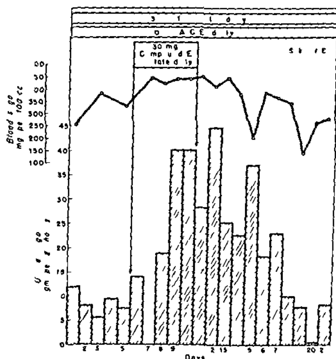


FIGURE 42 Increase in the level of the fasting blood sugar and in the urinary sugar during and after administration of cortisone acetate (compound E acetate) to a woman with coexisting Addison s disease and diabetes mellitus Urinary sugar was not determined on day 7 Reprinted by permission from Sprague R G Mason H L and Power M H Studies of the effects of adrenal cortical hormones on carbohydrate metabolism in human subjects *Proc Am Diabetes A (1949)* 9, 149 (1950)

ministered With this compound there was also a significant increase in urinary nitrogen

The third patient a young woman with both Addison s disease and diabetes in severe form was the subject of a more prolonged study of the effects of cortisone (Figure 42) The steroid was administered in a dose of 30 mg daily for five days in addition to basal treatment with a small dose of aqueous adrenal cortical extract while the diet and daily dose of insulin were kept constant There was a marked increase in glycosuria and some increase in the level of the fasting blood sugar during administration of cortisone (compound E) and for several days thereafter

Patients with coexisting Addison s disease and diabetes are obviously very sensitive subjects for the demonstration of the carbohydrate activity of steroids Although cortisone augments the severity of their diabetes it is possible to control the condition by administration of adequate amounts of insulin When this is done cortisone has a favorable effect on their clinical condition

Our limited experience with cortisone in relation to surgical procedures on patients with Addison s disease has already been mentioned However I should like to discuss briefly the use of cortisone in the preoperative and postoperative treatment in two types of cases in which temporary or permanent adrenal insufficiency may follow surgical treatment namely cases of hyperfunctioning lesions (tumor or hyperplasia) of the adrenal cortex associated with Cushing s syndrome and tumors of the anterior pituitary gland After experience with some thirty cases my associates and I are impressed with the fact that cortisone is highly effective as replacement therapy for patients with Cushing s syndrome who undergo surgical measures for removal of adrenal cortical tumors or for subtotal resection of hyperplastic adrenal glands Following surgical excision of the causative hyperfunctioning lesion of the adrenal cortex the patient with Cushing s syndrome must adjust himself to the profound and abrupt change from supernormal adrenal cortical function to subnormal function The effect on the appearance of a patient over a period of several months of this change in the level of adrenal cortical function is illustrated in Figure 45

Ralli Are those pictures of the same patient?

Sprague Yes Since there is evidence that most if not all of the features of Cushing s syndrome are attributable to overproduction of cortisone like steroids (6) it is not surprising that administration of cortisone should be helpful in the transition from a high to a relatively low level of adrenal function This has proved to be the case

The first need for cortisone in such patients is in the few days immediately following the operation for during this period the function of

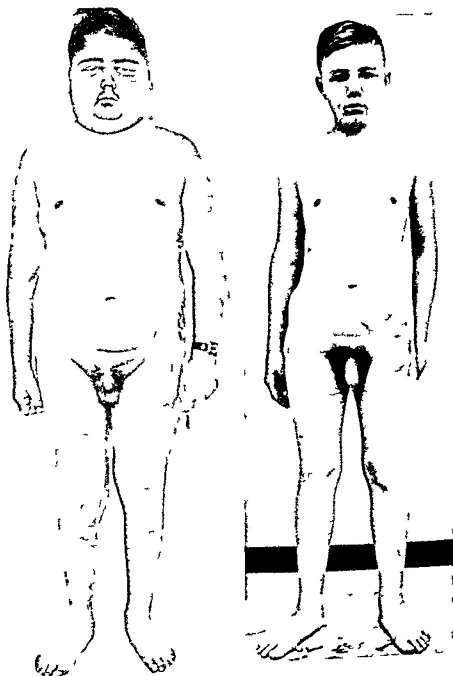


FIGURE 43 A boy age 14 years with Cushing s syndrome *(a)* before and *(b)* 11 months after subtotal adrenalectomy (Photograph on right courtesy of Dr M James Whitelaw Phoenix Arizona)

the remaining adrenal cortical tissue may be inadequate to withstand the stress of operation In the case of patients whose Cushing s syn drome is due to an adrenal cortical tumor the contralateral adrenal gland is atrophic and functionally depressed (Figure 44) In the case of patients with adrenal hyperplasia the function of the remnant left after subtotal adrenalectomy may be at least temporarily inadequate In both types of cases the administration of 200 mg of cortisone forty eight and twenty four hours before the operation and another 200 mg the morning of the operation has usually prevented the development of adrenal insufficiency in the immediate postoperative period A daily dose of 100 mg is continued for one to three days following the oper ation and is then gradually tapered off and discontinued

Bauer Parenterally?

Sprague Yes parenterally

We have been impressed that the postoperative course of patients who are treated in this way has been less eventful than was customary when aqueous adrenal cortical extract was used as preoperative and postoperative treatment presumably because larger amounts of the right kind of adrenal hormone are supplied when cortisone is admin istered

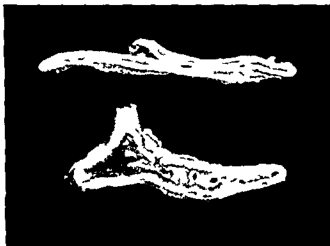


FIGURE 44 Atrophic right adrenal gland (above) from a woman with Cushing s syndrome due to a tumor of the left adrenal cortex com pared with a normal adrenal gland (below) Reprinted by permission from Kepler E J *et al* Adrenal cortical tumor associated with Cushing s syndrome report of case with metabolic studies and remarks on pathogenesis of Cushing s syndrome *J Clin Endocrinol* 8 499 (1948)

The results obtained by the use of cortisone in patients with Cushing's syndrome without adrenal cortical tumor before and after undergoing subtotal adrenalectomy are best illustrated by a comparison of the mortality figures for the eras before and after the use of cortisone The procedure of subtotal adrenalectomy as a treatment for Cushing's syndrome without tumor was first employed by us in the fall of 1945 and has now been carried out in a total of forty five cases A preliminary report of twenty nine of these cases has been made (7) In each case in which subtotal adrenalectomy has been performed the presence of an adrenal cortical tumor has been excluded by surgical exploration of one or both adrenals In the first twenty two cases preoperative and postoperative treatment consisted essentially of the administration of large doses of adrenal cortical extract In this group there were five hospital deaths and one death at home within a short time after the patient left the hospital or a total of six deaths in twenty two cases

Subsequently twenty three patients have been treated with cortisone before and after operation according to essentially the same plan as I have outlined for adrenal cortical tumors There was one death in this group of cases Furthermore we have been impressed that the postoperative course of these patients has been notably smoother than in the cases in which adrenal cortical extract was employed again probably due to the fact that larger amounts of cortisone like steroids were supplied in the form of cortisone than could be supplied in the form of aqueous adrenal cortical extract We feel that the improved results in these cases since the use of cortisone as preoperative and postoperative treatment are due chiefly to cortisone rather than to other factors

There has been another aspect of the care of these patients in which cortisone has been useful We have noted that starting about two to three weeks after completion of subtotal adrenalectomy a peculiar reaction consisting of nausea anorexia profound weakness lymphocytosis and in some instances hypercalcemia developed in the majority of the patients who ultimately obtained satisfactory remission of Cushing's syndrome There is not necessarily any disturbance of the plasma electrolytes We are unable to explain this reaction except to say that it is associated with a radical decrease in the level of adrenal cortical function from a supernormal level down to a subnormal level The condition has not been improved by large doses (100 to 200 ml daily) of adrenal cortical extract but it is improved rapidly and dramatically by 100 mg or less of cortisone daily

Cortisone has also been used as part of a program of end organ replacement therapy in cases of pituitary insufficiency Our usual program of treatment for patients with pronounced pituitary insufficiency has included the use of desiccated thyroid cortisone and the appropri

ate gonadal hormone that is testosterone in the male and estrogenic hormone in the female We have the impression that cortisone has been an important addition to the therapy of these patients Their general vigor health and appetite have been increased and they have apparently been able to withstand the stresses of ordinary living better than before the use of cortisone As is true in Addison s disease a clinical improvement due to cortisone does not seem to be intimately related to any laboratory or metabolic measurements which we have made Inasmuch as most patients who have pituitary insufficiency have sufficient residual adrenal cortical function to maintain electrolyte bal ance we have not usually found it necessary to use desoxycorticosterone in addition to cortisone

In the neurosurgical treatment of approximately ten patients with pituitary tumor we have administered cortisone before and after op eration While it is still too early to make any general statements con cerning the benefits of cortisone in these cases it has seemed to us that the postoperative course has been smoother than in similar cases man aged without cortisone One of the patients treated with cortisone sur vived an episode of purulent meningitis following the neurosurgical procedure It seems to be a reasonable assumption that many such patients regardless of the state of their pituitary function prior to op eration are at least temporarily hypophysectomized at the time of neurosurgical removal of the pituitary tumor The use of cortisone or ACTH as a means of enabling them to withstand the stress of the surgical procedure at a time when their own pituitary adrenal mecha nism is not operating normally would seem to be rational

Perera I should like to raise the question Dr Sprague as to whether 25 mg of cortisone in an Addisonian patient represents replacement or an excess of cortisone In other words does that dose of steroid result in a euadrenal or hyperadrenal subject? In my opinion the patient with Addison s disease requires smaller doses and even 25 mg may give rise on occasion to mental symptoms as well as to other manifestations of hyperadrenalism In a patient with both Addison s disease and rheuma toid arthritis we have observed dramatic relief of the arthritis following 25 mg of cortisone daily Perhaps the normal subject when given corti sone depresses his own endogenous cortisone formation beyond the contribution provided by needle or by mouth

Sprague I presume there is variation from one patient to another in what would be regarded as a replacement dose

What other signs of cortisone excess in Addisonian patients receiving only 25 mg a day have you observed?

Perera We have seen them infrequently but they were genuine and included alterations in facial contour excess hair growth as well as the expected metabolic changes

Sprague That is very interesting To the best of my knowledge we have not seen such things

Pincus May I ask if the cerebral effects may be antagonized by DCA?

Sprague We have wondered about that having followed the work of Dr Sayers and his associates on the antagonistic effects of cortisone and DCA on the electroshock seizure threshold in rats Unfortunately, we have not made any pertinent observations in human subjects Perhaps Dr Thorn has observed something

Thorn Our experience corresponds with that of Dr Sprague Of course our patients are maintained on extremely small doses of desoxycorticosterone namely 2 5 mg or less per day I do not believe that the desoxycorticosterone supplement has any appreciable influence on the brain metabolic changes

Sprague I think Dr Sayers has had to use relatively large doses to produce these changes as compared with the doses that are commonly used for human beings

Sayers We estimate the rats are absorbing about 1 2 mg of desoxycorticosterone acetate per day The animals are receiving in addition 2 mg of cortisone per day The dose of cortisone is in the same range as that employed in therapy in man On the other hand the dose of desoxycorticosterone acetate is much higher than that given to man DCA alone decreases brain excitability cortisone alone increases brain excitability The combination results in an increase in excitability much less than that induced by cortisone alone

What we need of course are more observations at various dose levels of DCA and cortisone in combination We still do not know just how many milligrams of cortisone are required to antagonize so many milligrams of DCA Our present results indicate that 2 mg of cortisone is more than enough to antagonize 1 2 mg of DCA As Dr Thorn points out Dr Sprague is using 2 5 mg of DCA and 25 mg of cortisone If the antagonism is on a molar basis then I would suspect that Dr Sprague is observing an almost pure cortisone like effect

Conn Dr Sprague our experience has been similar but not exactly the same Twenty five mg of cortisone has been too much in every Addisonian that we have had and our average optimum dose falls between 15 and 20 mg a day None of them have comfortably tolerated doses byond 20 mg a day But a peculiar thing for which I have no explanation and I should like to know Dr Thorn s observations in regard to it is that the few patients upon whom we have done total adrenalectomies we have found able to tolerate 25 and 50 mg a day without restlessness and jitteriness whereas the Addisonian is unable to do that

Thorn Our experience suggests that the majority of patients are able to tolerate 25 mg of cortisone daily This in most instances is given orally usually in divided doses of 12 5 mg in the morning and in the late afternoon or early evening We have encountered a few individuals who could not tolerate 25 mg of cortisone initially but subsequently after a preliminary period on 12 5 to 15 mg for a few days or a few weeks were able to tolerate 25 mg without difficulty We have several bilaterally adrenalectomized patients being maintained over prolonged period on 37 5 mg a day

Hechter I should like to ask whether the euphoria and hyperexcitability described with cortisone might be likened to a benzedrine reaction in some individuals

Sprague There are at least some superficial similarities between them

Hechter The view of Mann and Quastel (8) is that the central excitatory effects of benzedrine may be related to inhibition of amine oxidase in brain I was wondering whether cortisone exerts a similar effect on brain metabolism

Sprague As I say there is at least a superficial similarity between reactions to the two drugs However, I doubt that they are strictly comparable One thing that makes me think they are not is that there are so many variations in the cerebral response to cortisone in different patients which suggests that there may be differences in the mechanism of action of cortisone and benzedrine on cerebral function

Selye I was interested in Dr Perera s remark that Addisonians respond particularly readily to cortisone as regards the beneficial effect upon the rheumatoid arthritis Have you any observations concerning the sensitivity to cortisone in such a patient if at the same time you give DCA? Does that interfere with this particular sensitivity?

Perera That I cannot answer We employed various combinations of cortisone with DCA but for too short periods to make accurate comparisons of sensitivity as we were interested in the total effects on the patient

Sayers May I bring up a point here? It seems to me some of the effects we have been talking about would suggest that target cells can increase their tolerance to steroids For instance postoperatively the patient with adrenal hyperplasia requires very large doses of cortical steroids to maintain him The bilaterally adrenalectomized patient who was in a state of eucorticism before operation requires less maintenance therapy

Sprague I probably did not make it clear that the large doses I mentioned are used only immediately preceding and after operation Within a few days after subtotal adrenalectomy the doses of the pa

tients with Cushing's syndrome associated with adrenal cortical hyperplasia or tumor can be decreased greatly or discontinued

Sayers Postoperatively the patient with adrenal hyperplasia requires no greater quantity of cortical hormone for maintenance than the patient who was in a state of eucorticism before adrenalectomy?

Sprague Usually not with one possible exception I refer to the temporary need for cortisone for relief of symptoms of the postoperative reaction which I described previously This reaction and its relief by cortisone may be related in some way to the change from a high to a low level of adrenal cortical function in the patient with Cushing's syndrome

Astwood The reverse is true though isn't it that a patient after bilateral adrenalectomy requires a larger dose than a patient with Addison's disease?

Pincus Yes May I ask Dr Conn if the administration of compound B also leads to the hyperexcitability?

Conn No we have not noticed the hyperexcitability with B We have observed increased vim and vigor but not the irritability

Sayers That would agree with our studies in the rat Dr Dixon Woodbury has demonstrated that compound B given over long periods of time does not change electroshock threshold in the rat

Ralli Did you measure the capacity to handle water in any of the patients after they have been on cortisone?

Sprague We don't have very much information on that Dr Ralli When we have carried out the Robinson Kepler Power water test the results in Addisonian patients receiving cortisone have been variable At times the test has been restored to normal, and at other times it has not

The use of ACTH and cortisone in doses beyond the physiologic range (i e the pharmacologic range) in patients with intact adrenals is far more important than the use of these hormones as replacement therapy There is no question that cortisone and ACTH give impressive therapeutic responses in a variety of conditions for which there was formerly no good treatment on the other hand the overall picture with respect to the therapeutic use of these agents in nonhormonal conditions calls for somewhat more conservatism than was apparent in the newspaper reports in 1949 and 1950 Accumulated clinical experience has shown that it is frequently necessary to be content with a therapeutic result which is something less than ideal in order to avoid important undesirable effects It is encouraging to note however that something is being learned about how to use the hormones effectively and safely

As previously stated my own personal experience with cortisone and ACTH has been limited largely to states of adrenal cortical insuf

ficiency In what follows I should like to present in brief form some appraisals by various members of the staff of the Mayo Clinic of the clinical usefulness of these agents in several different conditions I shall not attempt to cover the entire field of their clinical usefulness

In the use of cortisone in the treatment of rheumatoid arthritis at the Mayo Clinic the trend in the past year has been toward the oral use of the hormone in doses which are small enough to avoid undesirable effects while producing a worthwhile clinical response This would seem to be a logical method of attacking the problem of undesirable effects while trying to secure significant suppression of rheumatoid arthritis The data which follow are based upon a report by Ward Slocumb Polley Lowman and Hench (9) concerning oral administration of cortisone acetate to one hundred patients with rheumatoid arthritis

In ninety nine of the one hundred patients studied by these authors oral administration of cortisone produced an effective antirheumatic response One patient did not respond to cortisone given orally but subsequently responded to intramuscular administrations An estimate of the clinical response in seventy three cases in which cortisone was given only by mouth is presented in Table XIX In about two thirds of the patients very marked or marked relief of symptoms was obtained In the remaining third with the exception of the one patient who did not respond relief was estimated as moderate

The daily dose of cortisone given orally to bring about an initial suppression of symptoms varied greatly in different cases depending in part upon the severity of the disease (Table XX) In some cases doses as high as 200 mg daily were employed and in others doses as

TABLE XIX

Results in 73 Cases of Cortisone Given in Oral Form Only

Relief of Symptoms	Cases: Number	Cases: Per cent
Very marked (90 100 per cent)	19	26
Marked (75 89 per cent)	28	39
Moderate (50 74 per cent)	25	34
None	1	1
Total	73	100

Reprinted by permission from Ward, L. E., et al.: Clinical effect of cortisone administered orally to patients with rheumatoid arthritis. Proc. Staff Meet. Mayo Clin. 26: 361 (1951)

TABLE XX

Doses Used Orally to Bring Symptoms Under Control Initially 72 Cases

Daily Dose mg	Cases Number	Cases Per cent
200 0	3	4
100 0	47*	65
75 0	6	8
50 0	14	20
37 5	2	3
Total	72	100

S x of these patients rece ved 300 mg on the fi st d y and 100 mg thereafter

Repr nted by perm ss on f om Ward L E *et al* Cl l effect of cortiso e adm tered orally to patients with rheumatoid arthritis *Prof Staff Meet M yo Cl i* 26 *361 (1951)*

low as 37 5 mg daily sufficed As the study progressed it became ap parent that many of those receiving 100 mg or more early in the course of study could have been started on smaller doses After initial sup pression of the symptoms of the disease and without regard to the sedimentation rate the dosage of cortisone was reduced gradually Re duction of dosage was begun as soon as moderate relief of symptoms occurred

The daily doses of cortisone which were used orally to maintain im provement obtained initially by the use of larger doses are given in Table XXI It will be noted that the daily maintenance dose ranged from as little as 25 mg to as much as 100 mg however the majority of the seventy two patients in this group were maintained on doses of 62 5 mg daily or less

It would be anticipated that the use of the smallest possible daily dose of cortisone would minimize the occurrence and severity of un desirable physiologic effects This proved to be the case As a rule the undesirable effects which were observed in these patients were mild and not harmful It is of interest that it was not necessary to discon tinue the use of cortisone in any case in the group because of undesired effects The necessity for discontinuing cortisone was avoided by re duction of dosage Undesirable physiologic effects occurred in fifty four of the one hundred cases

Bauer Did the majority of these undesirable effects occur in patients receiving the larger doses?

TABLE XXI

Doses Employed Orally to Maintain Improvement 72 Cases

Daily Dose mg	Cases	
	Number	Per cent
100 0	9	13
87 5	1	1
75 0	11	15
62 5	13	18
50 0	23	32
37 5	13	18
25 0	2	3
Total	72	100

Reprinted by permission from Ward L E et al Clinical effects of cortisone administered orally to patients with rheumatoid arthritis *Proc Staff Meet Mayo Clin* 26 361 (1951)

TABLE XXII

Influence of Dose on Incidence of Side Effects

Daily Dose	Side Effects per cent of patients
75 mg or more	63
Less than 75 mg	21

Reprinted by permission from Ward L E et al Clinical effects of cortisone administered orally to patients with rheumatoid arthritis *Proc Staff Meet Mayo Clin* 26 361 (1951)

Sprague Yes there was a relationship between the size of the daily dose and the occurrence of undesirable physiologic effects (Table XXII) Thus almost two thirds of the patients who received doses of 75 mg or more daily experienced undesirable effects on the other hand only about a fifth of the patients who received doses less than 75 mg daily had undesirable effects

In Table XXIII are given some data on the influence of sex on the incidence of undesirable effects in one hundred cases These data seem to confirm our early impression that women are more susceptible to such effects than are men and this seems to apply particularly to post menopausal women

TABLE XXIII

Influence of Sex on Incidence of Side Effects 100 Cases

Group	Side Effects per cent of patients	
	Daily Doses 75 mg or more	Daily Doses less than 75 mg
Men	50	5
Premenopausal women	72	24
Postmenopausal women	85	31

Reprinted by permission from Ward L E *et al* Clinical effects of cortisone administered orally to patients with rheumatoid arthritis *P of St ff Meet Mayo Clin 26 361 (1951)*

The study just cited lends support to the concept that in rheumatoid arthritis prolonged systematic use of cortisone with reduction of the daily dosage to a level which is well tolerated physiologically will yield *beneficial suppression of active rheumatoid arthritis in many patients* and that the incidence of undesirable physiologic effects will be low

I should like to comment briefly on one physiologic effect of cortisone which is of considerable potential clinical importance namely adrenal suppression A single case which recently came to my attention illustrates the point A man with rheumatoid spondylitis was treated with cortisone for approximately six months It was then found necessary to carry out an orthopedic operation on his spine Cortisone was withdrawn shortly before the operation was to be done About thirty six hours after completion of the operation the patient went into a state of shock and his blood pressure was unobtainable He died and at necropsy was found to have atrophic adrenals His death was presumably attributable to inadequate adrenal function under a condition of stress

The developments of the past year in the field of hematology with respect to the use of cortisone and ACTH have dealt chiefly with the so called hypersplenic syndromes It is an understatement to say that use of these drugs in this field has resulted in a state of confusion largely due to the fact that there seems to be no uniformity of response of these conditions to administration of the hormones For example there are reports of benefit in cases of splenic neutropenia However one such patient treated with cortisone at the Mayo Clinic did not respond The condition usually is improved by splenectomy In cases of thrombocytopenic purpura responses to the hormones have varied from dramatic to none at all Here again splenectomy is frequently an effective form of therapy Likewise the results in cases of acquired hemolytic icterus

have been variable In some instances extremely favorable clinical responses have been observed and in others there has been no response The variable results in acquired hemolytic icterus suggest that perhaps cases which are lumped together under this heading actually represent a group of conditions having different underlying mechanisms

It is now well established that cortisone and ACTH are capable of bringing about dramatic symptomatic improvement in patients with acute systemic lupus erythematosus one of the so-called collagen diseases Such acute manifestations as fever prostration anorexia articular pain and lesions of the skin and mucous membranes may all subside under the influence of the hormones However such remissions of acute lupus are temporary and evidences of relapse usually begin to make their appearance with a few weeks As the disease progresses therapy with the hormones becomes less effective and the termination is fatal in many instances My colleague Dr L A Brunsting tells me that of seven patients having acute disseminated lupus erythematosus and treated with cortisone or ACTH all except one are now dead within a period of two years In reviewing the literature dealing with the use of cortisone and ACTH in acute lupus one gets a distinct impression that undesirable physiologic effects of one sort or another are particularly common probably due to the widespread involvement of various tissues of the body by the disease process

In the case of periarteritis nodosa another collagen disease with an extremely serious prognosis the situation seems to be moderately encouraging as judged by the experience of Dr R M Shick and his associates They have had the opportunity to administer cortisone or ACTH or both to sixteen patients with periarteritis nodosa In each instance the clinical diagnosis was confirmed histologically by biopsy In all cases there has been prompt symptomatic improvement after administration of the hormones Partial relapses occurred in a few cases after withdrawal of the hormones followed by improvement when treatment was resumed Three patients improved initially and subsequently died in cardiorenal failure At necropsy complete healing of all arterial lesions was found however there was extensive fibrous obliteration of the lumens of the involved vessels resulting in widespread visceral infarction One other patient is still receiving continuous suppressive therapy because it was previously observed that relapse occurred after withdrawal of the hormones In twelve cases the disease was apparently in remission when the patients were last observed from two months to almost two years after treatment although some evidence of residual vascular damage has persisted in most instances Most patients showed evidence of hormonal excess during treatment but this disappeared after withdrawal of the hormones On the basis of this

experience it would appear that cortisone and ACTH are capable of inducing clinical remission in periarteritis nodosa and may suppress the primary pathologic lesion and permit it to heal Since spontaneous remissions of this disease seem to occur infrequently it would appear that this form of treatment of periarteritis nodosa offers far more than any other form of treatment previously available In a conversation with Dr Bauer last night he suggested that prolonged remissions of periarteritis nodosa after stopping administration of cortisone or ACTH may indicate that the disease has been eradicated and that the inciting agent whatever it may be is no longer present

The foregoing represents a brief summary of therapeutic results obtained in a few of the conditions in which the hormones cortisone and ACTH have been applied These results are presented with the purpose of giving a conservative overall view of what can be expected in specific conditions when cortisone and ACTH are used It should be emphasized that probably no cures are effected the inflammatory diseases of mesenchymal tissue are suppressed by the hormones but usually recur when the hormones are withdrawn

I might take another moment to list some of the general problems in this field

a) The question of long term evaluation of these agents in terms of good and bad effects on human beings

b) The question of mechanism of action of the adrenal hormones in inflammatory and allergic conditions

c) The problem of control of undesirable effects I personally feel that the most reliable weapon here is the type of procedure employed by Ward and his associates in the case of rheumatoid arthritis namely reduction of dosage to the lowest possible level There are of course other things that can be done for certain undesirable effects but there is no need to discuss these now

d) The use of these hormones as tools for investigation of the underlying mechanism of the diseases which they favorably affect

e) Then lastly the whole question of other compounds both steroidal and nonsteroidal which might have therapeutic activity similar to that of cortisone and ACTH The efforts which have been made thus far to attack this problem have not been fruitful That doesn t mean that the possibilities are exhausted

Sayers Would you expand on the last point? Is it the availability of steroids or the difficulties of assessing the therapeutic effectiveness of these compounds in man?

Sprague Both Although many steroids have been made available for clinical trial there is no convincing evidence that any of them with the exception of compound F duplicate the therapeutic and other phy

siologic effects of cortisone New compounds used to treat human beings must be assessed with great care and objectivity

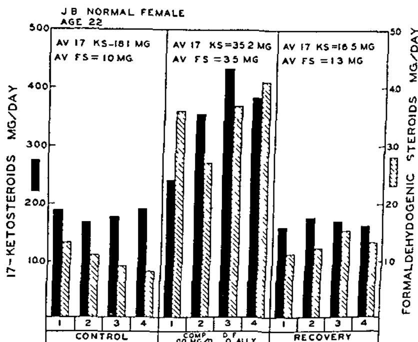
Pincus There has been a great deal of data given here that compounds B and F appear to be adrenal secretory products What is the situation in regard to their clinical effect?

Sprague I can describe our observations briefly because they are quite limited We have given 500 mg of compound B intramuscularly daily for eighteen days to a patient with rheumatoid arthritis a total of 9 gm of compound B It had no detectable effect on the arthritis The principal effect that was observed was the electrolyte effect

Long Dr Conn might have a point to make on compound B

Conn I reported most of our metabolic data on compound B at this Conference last year I should like to bring to your attention some of our recent data on urinary excretion of 17 ketosteroids and of formaldehydogenic steroids when compounds F B E and S are given orally and intramuscularly In addition I should like to show some of our data on the metabolic effects in man of free compound F and compound F acetate when each is given either orally or intramuscularly in the same individual I believe these data bear importantly on some of the deliberations and discussions presented by Dr Nelson Dr Hechter and Dr Pincus You will recall that Dr Pincus brought up the question of the possible role of the liver in the metabolism of the steroids themselves He indicated that the isolated liver perfused with compound F removes the latter at a very rapid rate Dr Hechter stated that perfusion of the liver with a given steroid results in a very rapid transformation of the administered steroid to as many as 10 or 20 different compounds which have not yet been identified Dr Nelson pointed out that the liver seems to remove steroids rapidly from the blood as indicated by the large arteriovenous difference in compound F like material across the liver He did not find this to be the case when he measured arteriovenous differences across the kidney

About a year ago we discovered that when a steroid is administered orally to a normal man there results a sharp rise in urinary excretion of 17 ketosteroids When the same dose of the same compound is given to the same individual intramuscularly however there occurs either no rise or an actual fall of 17 ketosteroid excretion We regard the increase in 17 ketosteroid excretion after oral administration to be due most likely to a transformation in the liver since upon oral administration the portal circulation brings the steroid in high concentration to the liver May I now show some of these experiments in which we have determined both 17 keto and the formaldehydogenic steroid excretions in normal people when compounds F E B and S have been given both orally and intramuscularly



EFFECT OF ORAL FREE COMPOUND F ON URINARY STEROIDAL EXCRETION

FIGURE 45

Figure 45 shows the results obtained when a normal young female was given 400 mg per day *orally of compound F as the free alcohol* Note the sharp rise in both 17 ketosteroid and formaldehydogenic steroid excretion and its rapid return to baseline values upon cessation of administration

I might indicate at this point that oral compound S in similar dosage results in just as great a rise of 17 ketosteroid excretion but in so significant elevation of formaldehydogenic steroid excretion It is noteworthy too that we observed no evidence of metabolic activity of the administered compound S If the adrenal gland is capable under physiological circumstances of converting S to F and if a sufficient quantity of unaltered S left the liver to be converted to compound F at least there were no metabolic activities to suggest that compound F was active

Figure 46 shows the effect on urinary excretion of steroidal materials when the 400 mg per day of free compound F is given intramuscularly instead of orally The 17 ketosteroids are initially depressed and then rise mildly but as compared with the rise following oral administration this rise is insignificant On the other hand excretion of formaldehydogenic steroids rose sharply

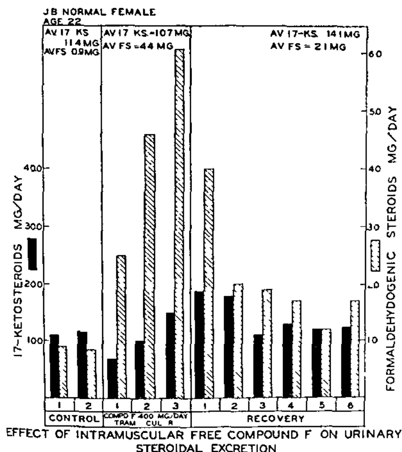
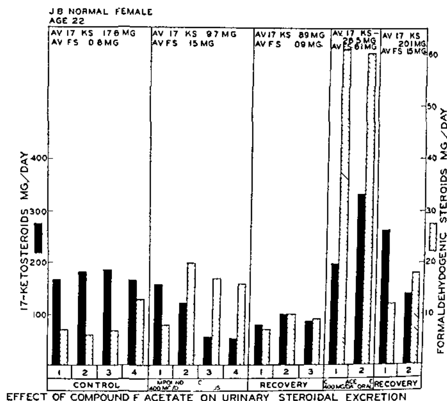


Figure 46

Figure 47 shows that when 400 mg per day of the acetate of F are given intramuscularly there occurs a depression of adrenal cortical function as indicated by the fall of 17 ketosteroids There is also a mild rise in the urine of formaldehydogenic steroids Both of these findings indicate that some of the F acetate has been absorbed Enough has been absorbed to inhibit endogenous ACTH production but not enough to give important overall metabolic effects as is shown in subsequent Figures

It would appear to me that this depression of adrenal function produced by compound F acetate intramuscularly together with the absence of significant metabolic effects of the administered steroids probably explains the peculiar phenomenon which Dr Nelson reported yesterday You may recall that he observed a precipitous fall in the



EFFECT OF COMPOUND F ACETATE ON URINARY STEROIDAL EXCRETION

FIGURE 47

concentration of 17 hydroxycorticoids in blood when he administered compound F acetate intramuscularly

Again in Figure 47 note the tremendous difference in steroid excretions when compound F acetate is given orally instead of intramuscularly At this point I should remark that compound F acetate orally is extremely active metabolically

Tables XXIV and XXV are self explanatory and show the same phenomena that I have described for oral versus intramuscular compound F It should be noted that the doses of cortisone and of compound B used in these studies were 200 mg per day while in the compound F studies doses of 400 mg per day were employed While the changes in 17 ketosteroid excretions upon oral administration of these compounds versus their intramuscular administration are not as large as those observed in the compound F studies they nevertheless move in the same directions

Sayers You observed an increase in both?

Conn Yes in both when these compounds were given orally

Let us consider some of the results which we have obtained with

TABLE XXIV

Effect of Cortisone Acetate 200 mg./day on Urinary Steroidal Excretion in Normal Males (R.S. and G.A.)

Route of Administration	17 Ketosteroids mg./day			Formaldehydogenic Steroids mg./day		
	Control Period	Steroid Administration	Recovery Period	Control Period	Steroid Administration	Recovery Period
Intramuscular (R.S.)	16.0(5)	12.7(10)	11.6(16)	1.5(6)	2.10(10)	1.9(16)
Oral (G.A.)	15.9(3)	19.4(5)	11.6(3)	1.2(3)	1.7(5)	0.9(1)

TABLE XXV

Effect of Corticosterone (Compound B) 200 mg./day on Urinary Steroidal Excretion in a Normal Male (G.A.)

Route of Administration	17 Ketosteroids mg./day			Formaldehydogenic Steroids mg./day		
	Control Period	Steroid Administration	Recovery Period	Control Period	Steroid Administration	Recovery Period
Intramuscular	15.8(4)	13.9(6)	13.5(2)	1.3(4)	1.9(6)	1.8(2)
Oral	13.4(5)	16.5(5)	13.0(4)	1.0(5)	1.5(5)	1.1(4)

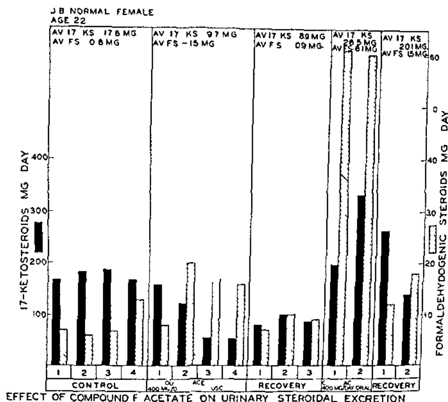


FIGURE 47

concentration of 17 hydroxycorticoids in blood when he administered compound F acetate intramuscularly

Again in Figure 47 note the tremendous difference in steroid excretions when compound F acetate is given orally instead of intramuscularly At this point I should remark that compound F acetate orally is extremely active metabolically

Tables XXIV and XXV are self explanatory and show the same phenomena that I have described for oral versus intramuscular compound F It should be noted that the doses of cortisone and of compound B used in these studies were 200 mg per day while in the compound F studies doses of 400 mg per day were employed While the changes in 17 ketosteroid excretions upon oral administration of these compounds versus their intramuscular administration are not as large as those observed in the compound F studies they nevertheless move in the same directions

Sayers You observed an increase in both?

Conn Yes in both when these compounds were given orally

Let us consider some of the results which we have obtained with

compound S acetate administered both orally and intramuscularly Figure 48 shows the results obtained on a normal man when 200 mg per day of compound S acetate were given orally A great rise of 17 ketosteroid excretion occurred A small and probably insignificant rise in excretion of formaldehydogenic steroids also occurred When the same dose of compound S was given intramuscularly no elevation of 17 ketosteroid excretion occurred and the mild rise in urinary formal dehydogenic steroids persisted No significant metabolic effects were observed from compound S acetate when administered by either route

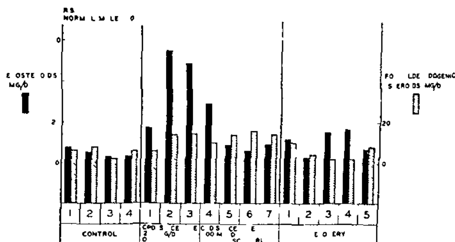
Figure 49 illustrates the results obtained in another normal male This time 400 mgs per day of compound S acetate was given orally There was no significant increase in formaldehydogenic steroids but 17 ketosteroids again rose to very high levels The same dose was given intramuscularly for just one day and no significant increase in either of these urinary excretory products was observed Again no metabolic effects!

Figure 50 shows that precisely the same results are obtained when compound S acetate is given orally to a patient with Addison s disease Thus it is clear that the normally functioning adrenal cortex is not necessary in order for 17 ketosteroid excretion to rise sharply after the oral administration of compound S acetate On the other hand when one repeats this experiment on a patient with liver disease (Figure 51) a much smaller increase in urinary 17 ketosteroids is found In subject E J the increase in 17 ketosteroids was one third of that observed in the normals and in the Addisonian and in subject G B (Figure 52) the increase was one fifth of that observed in the subjects with normal hepatic function

Thorn What degree of hepatic impairment was there in these patients?

Conn I cannot remember the exact figures of the liver function tests but there was marked impairment

Thorn The reason I asked the question Dr Conn was to bring out the difference between cirrhosis with and without severe hepatocellular failure I *think it makes considerable difference whether or not the* patients with cirrhosis have been demonstrated to have marked hepato cellular impairment We have been particularly interested in the in termediary metabolism of adrenal steroids when administered to patients with known severe acute hepato intoxication such as occurs with carbon tetrachloride The interesting thing which we observed in these patients was that 500 mg of cortisone given daily caused no in crease in 17 ketosteroid excretion but did produce a marked rise in the 11 oxysteroid excretion in the urine This would fit with the ideas which have been expressed We are rechecking the effect of administer



EFFECT OF COMPOUND S ACETATE ON URINARY STEROIDAL EXCRETION

Figure 18

EFFECT OF COMPOUND S ACETATE ON URINARY STEROIDAL EXCRETION

Figure 49

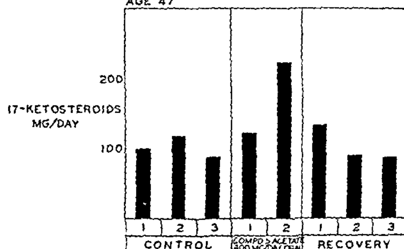


FIGURE 51

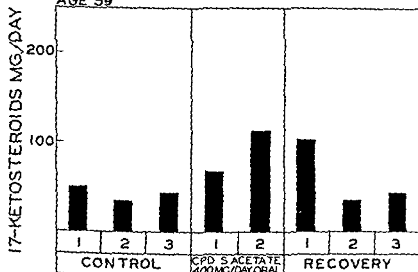
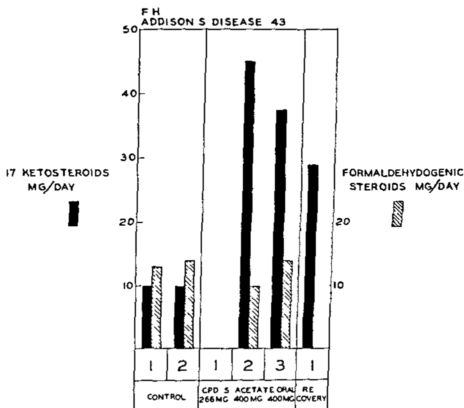
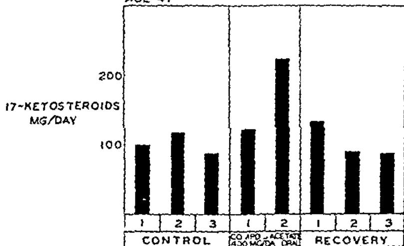


FIGURE 52



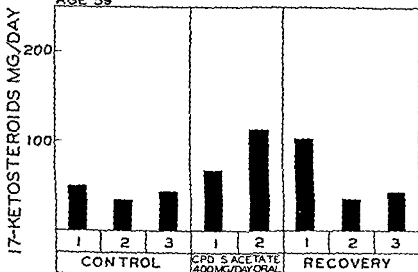
EFFECT OF COMPOUND S ACETATE ON URINARY STEROIDAL EXCRETION

FIGURE 50



EFFECT OF COMPOUND S ACETATE ON RENAL EXCRETION OF 17-KETOSTEROIDS

FIGURE 51



EFFECT OF COMPOUND S ACETATE ON RENAL EXCRETION OF 17-KETOSTEROIDS

FIGURE 52

ing the same dose of steroid to these patients when they have recovered from their liver impairment

Conn I should now like to show some of our studies with compound F In view of the predominance of compound F among the steroidal compounds which emerge from the ACTH stimulated adrenal gland, it was somewhat surprising that initial reports on the metabolic effect in man of intramuscularly administered compound F acetate indicated very minor effects It was therefore decided to study the metabolic effects of the larger doses of compound F I wish to demonstrate that *active* compound F has extremely intense effects on all of the metabolic processes which we have been able to study At the same time we can confirm the earlier observations that intramuscularly administered compound F acetate is relatively inadequate metabolically The failure to observe significant metabolic effects was not due to the matter of dosage

In four separate balance studies, we have determined the comparative metabolic effects of compound F as the free alcohol, and of compound F acetate each given both orally and intramuscularly All of the studies were done on the same normal subject in order to eliminate possible differences among individuals in their responses to compound F For comparative purposes a fifth study was carried out on the same subject this time with cortisone acetate given orally In all of these experiments the dose of each compound was 400 mg per day

I should like to demonstrate first the true metabolic effects of compound F when this substance is free to exert them Figure 53 shows the effects upon electrolyte metabolism of 400 mg per day of free compound F administered orally One observes an initial potassium diuresis along with a marked renal retention of sodium and chloride Both of these phenomena reverse themselves in the recovery period There occurred also a 4 kilogram increase in body weight along with a sharp decrease of the hematocrit reading These effects upon electrolyte metabolism are precisely those that we have come to associate with 11 desoxycorticosterone

In this same experiment the effects of this compound upon organic metabolism are shown in Figure 54 Note the rise of the fasting blood sugar the fall of the eosinophils to zero the increase in uric acid excretion the sharply negative nitrogen balance and the production of significant glycosuria

Figure 55 demonstrates the effect upon glucose tolerance of four days of free compound F orally at a dose level of 400 mg per day I have already shown in Figure 45 the urinary excretions of 17 ketosteroids and of formaldehydogenic steroids during this experiment Thus all of the metabolic changes which we have observed during oral adminis

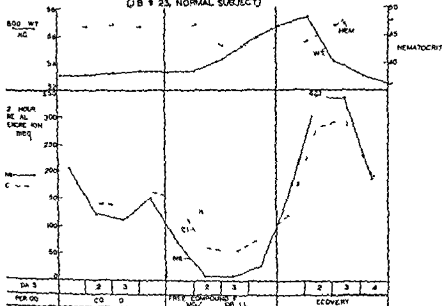


FIGURE 53

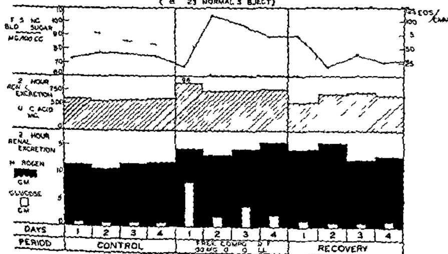


FIGURE 54

EFFECT OF COMP F (17-HYDROXYCORTICOSTERONE) UPON GLUCOSE TOLERANCE

(J B ♀ 22-NORMAL SUBJECT)

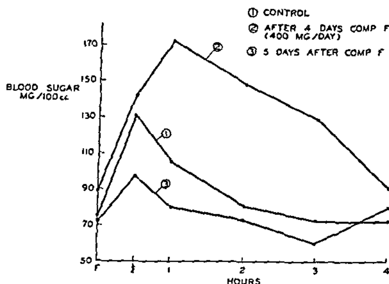


FIGURE 55

tration of free compound F are those which occur when normal people are given large doses of ACTH

Figure 56 demonstrates some of the interesting differences in meta bolic effect between free compound F and compound F acetate Note that the two control periods are in good agreement When compound F acetate is given intramuscularly the effects upon electrolyte meta bolism if present at all are extremely mild However when the same compound is given in the same dosage orally the effects upon electro lyte metabolism are intense When the free alcohol of compound F is given intramuscularly the effects are quite intense Further when the free compound is given orally it exerts its greatest effects upon electrolyte metabolism In the last column cortisone acetate orally is compared with F acetate orally given in the same dosage It is clear that cortisone has less effect upon electrolyte metabolism than does compound F

Pincus Is it not possible that a metabolite arising from the liver from cortisone or F might have a specific effect on the synovia?

Conn Yes indeed I believe that is possible

Pincus If that is the case I would like to ask about rheumatoid arthritis in people with cirrhosis of the liver

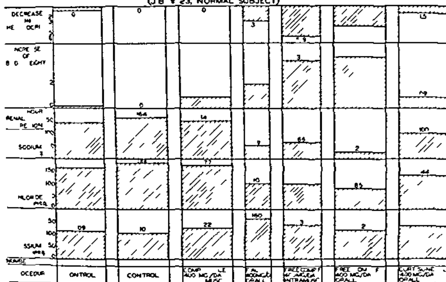


FIGURE 56

COMPARATIVE METABOLIC EFFECTS OF FREE AND ACETYLATED COMP F (ORALLY AND IM) A D OF CORTISONE ACETATE ORALLY DOSE-400MG/DAY OF EACH COMPOUND (JB # 23 NORMAL SUBJECT)

PROCEDURE # OF DAYS	CONTROL 4	CONTROL 4	F-AC 4 (IM)	F-AC 2 (O)	FREE F 3 (IM)	FREE F 4 (O)	CORTISONE AC 4 (ORAL)
BLOOD SUGAR mg %	74	76	79	104	107	95	97
EOSINOPHILS	72	67	42	0	2	0	4
24 HOUR RENAL EXCRETION							
NITOGEN gms	11 3	11 0	10 8	14 0	15 0	14.3	15 8
GLUCOSE gms	0 6	0 6	1 0	5.2	4 9	3 7	1 2
URIC ACID mg	575	581	645	787	867	800	797
17-KS mg	18 1	17 6	9 7	26 5	10 7	35 2	22.6
FORMALD S mg	1 0	0 8	1.5	6 1	4 4	3 5	4 5

FIGURE 57

EFFECT OF COMP F (17-HYDROXYCORTICOSTERONE) UPON GLUCOSE TOLERANCE

(J B ♀ 22-NORMAL SUBJECT)

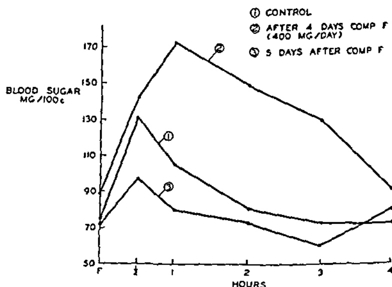


FIGURE 55

tration of free compound F are those which occur when normal people are given large doses of ACTH

Figure 56 demonstrates some of the interesting differences in metabolic effect between free compound F and compound F acetate Note that the two control periods are in good agreement When compound F acetate is given intramuscularly the effects upon electrolyte metabolism if present at all are extremely mild However when the same compound is given in the same dosage orally the effects upon electrolyte metabolism are intense When the free alcohol of compound F is given intramuscularly the effects are quite intense Further, when the free compound is given orally it exerts its greatest effects upon electrolyte metabolism In the last column cortisone acetate orally is compared with F acetate orally given in the same dosage It is clear that cortisone has less effect upon electrolyte metabolism than does compound F

Pincus Is it not possible that a metabolite arising from the liver from cortisone or F might have a specific effect on the synovia?

Conn Yes, indeed I believe that is possible

Pincus If that is the case I would like to ask about rheumatoid arthritis in people with cirrhosis of the liver

COMPARATIVE EFFECTS UPON ELECTROLYTE METABOLISM OF FREE AND ACETYLATED COMP F (ORALLY AND INTRAMUSCULARLY) AND OF CORTISONE ACETATE ORALLY DOSE 400 MG/DAY OF EACH COMPOUND (J B ♀ 23 NORMAL SUBJECT)

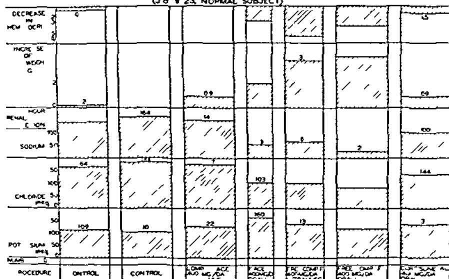


FIGURE 56

COMPARATIVE METABOLIC EFFECTS OF FREE AND ACETYLATED COMP F (ORALLY AND IM) AND OF CORTISONE ACETATE ORALLY DOSE–400MG/DAY OF EACH COMPOUND (J B ♀ 23 NORMAL SUBJECT)

PROCEDURE # OF DAYS	CONTROL 4	CONTROL 4	F–AC 4 (IM)	F–AC 2 (O)	FREE F 3 (IM)	FREE F 4 (O)	CORTISONE AC 4 (ORAL)
BLOOD SUGAR mg%	74	76	79	104	107	95	97
EOSINOPHILS	72	67	42	0	2	0	4
24 HOUR RENAL EXCRETION							
NITOGEN gms	11 3	11 0	10 8	14 0	15 0	14.3	15 8
GLUCOSE gms	0 6	0.6	1 0	5.2	4.9	3 7	1 2
URIC ACID mg	575	581	64.5	787	867	800	797
17–KS mg	18 1	17 6	9 7	26 5	10 7	35 2	22 6
FORMALD S mg	1 0	0 8	1.5	6 1	4 4	3 5	4 5

FIGURE 57

Ralli You rarely see rheumatoid arthritis in patients with cirrhosis of the liver

Bauer We have seen it but I can only recall two cases Therefore one cannot generalize

Ralli You would agree it was exceedingly rare We see many cases of cirrhosis of the liver at Bellevue Hospital they are mostly alcoholics and perhaps that is what is preventing the arthritis

Conn Figure 57 compares the effects upon organic metabolism of these compounds by the various routes of administration The chart is set up in the same way as Figure 56 but the columns have been eliminated Each number represents the average for the period just as do the numbers in Figure 56 Note that F acetate intramuscularly did not affect the fasting blood sugar level while the latter was raised in each of the other four experiments F acetate decreased the eosinophil count mildly when it was given intramuscularly but in the other four experiments the eosinophils fell essentially to zero F acetate intramuscularly failed to produce negative nitrogen balance while the latter was produced regularly in each of the other experiments With F acetate intramuscularly, urinary glucose rose very slightly There was a much greater effect upon urinary glucose from F acetate orally and from free compound F either orally or intramuscularly Cortisone had less effect in producing glycosuria than did the *active* compound F Urinary uric acid was increased slightly by intramuscular F acetate but it was increased greatly in each of the other four experiments I have already discussed the 17 ketosteroid excretion and the formaldehydogenic steroid excretion

Figure 58 shows the effects of these various compounds and their routes of administration upon glucose tolerance It will be observed that compound F acetate intramuscularly does indeed decrease carbohydrate tolerance but that compound F acetate orally or free compound F intramuscularly produce much greater decreases in carbohydrate tolerance

These compound F data can be summarized by stating that metabolic effects in man of *active* compound F include intense renal retention of sodium chloride and water potassium diuresis severely negative nitrogen balance great loss of carbohydrate tolerance with glycosuria urocosuria rapid hemodilution and the disappearance of circulating eosinophils In addition acne and moon face may occur within seventy two hours When given orally as either the acetate or the free compound there occurs a sharp increase in the urinary 17 ketosteroids and formaldehydogenic steroids

The most striking differences upon the metabolic processes between cortisone and compound F administered in the same dosage relate to the much more intense effects of compound F both upon electrolyte

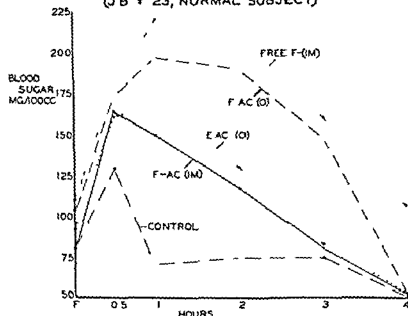


FIGURE 58

metabolism and upon carbohydrate metabolism the remaining meta bolic effects being of equal magnitude for both compounds

While free compound F given intramuscularly produces intense overall metabolic effects intramuscular compound F acetate is relatively inert Whether this is related wholly to differences in solubilities or to an inability of the body to deacetylate the compound remains to be de termined In any case investigators now engaged in experiments with compound F acetate should be aware of this fact since with compound F acetate the results of their experiments will be determined in large measure by its route of administration

Pincus What is your interpretation?

Conn As you have already indicated a good deal of investigation with respect to what happens to steroids as they traverse the liver will prove to be extremely important

Astwood Would Dr Conn or Dr Sprague have a suggestion as to where the large quantity of urinary ketosteroid comes from after the administration of corticotropin? The adrenal does not secrete into the portal system

Conn I m willing to stick my neck out I have a feeling from the results which we have obtained that the level of concentration of ste

roids in the portal circulation is related directly to the amount of 17 ketosteroids in the urine whether it follows administration of ACTH or whether it is after the administration orally or parenterally of a steroid Dr Nelson pointed out that the A V difference in steroid concentration was great across the liver but negligible across the kidney It is likely that ACTH produces a sharp even though transient elevation of adrenal steroid concentration in the systemic blood The hepatic blood flow shares importantly in this general systemic rise of steroid concentration and during this period rapid hepatic conversion to 17 ketosteroids is occurring

As a corollary to this one can say first that a large proportion of excreted 17 ketosteroids probably do not have an androgenic precursor normally and secondly that one can set up conditions such as with orally administered compound S in which a metabolically inert steroid gives rise to the excretion of enormous amounts of urinary 17 ketosteroids

Nelson The levels of 17 hydroxycortical steroids found in blood are no higher after ACTH administration than they are after giving oral E or F certainly, and in the case of intramuscular ACTH they are lower Nevertheless much higher levels of 17 ketosteroids are found in the urine after ACTH I should also point out that in the case of our isolations from the adrenal venous blood of the cow which we carried out with Dr Gassner we have found 17 ketosteroids or at least compounds which give the Zimmerman reaction coming directly from the adrenal gland

Conn Yes but that is not out of line with the idea I have suggested If the blood level after ACTH is similar to the blood level after oral administration of a steroid you would expect the 17 ketosteroid excretion to be about the same with both and where the blood level is low as with parenterally administered steroid you would expect the 17 ketosteroid excretion to be low as it is

Nelson But there are apparently 17 ketosteroids being secreted directly by the adrenal gland

Sprague It seems to me there is a dilemma here Orally administered cortisone presumably gets into the portal circulation goes through the liver and therefore stands a better chance of being degraded to 17 ketosteroids than if it were given parenterally But the cortisone structure is thought to be essential for antirheumatic activity The antirheumatic effects of cortisone given orally are approximately equal to the antirheumatic effects of cortisone given parenterally If the structure of cortisone is altered on its first trip through the liver why should not the orally administered cortisone lose a large part of its antirheumatic activity? Apparently it does not Dr H L Mason has suggested to me that the dilemma would be resolved if it were known

that absorption of cortisone from the gastrointestinal tract were largely via the lymphatics rather than via the portal circulation

Pincus I presented a paper on this point at the Mexican Symposium on Steroids (10) pointing out this difference between oral and parenteral cortisone

Conn I think another point is important in this connection namely that patients with cirrhosis of the liver as a group have lower 17 ketosteroid excretions than normal people and increased 11-oxysteroid excretions

White The next important step would be to treat a patient with cirrhosis of the liver and rheumatoid arthritis with cortisone or F by mouth Are there therapeutic effects?

Sprague I know of one patient treated with cortisone given intramuscularly It was effective It was interesting that ketosteroids entirely disappeared from his urine when he received cortisone Perhaps his own adrenal cortices were suppressed and he was not capable of converting any of the administered cortisone to 17 ketosteroids

Thorn I should like to ask Dr Bauer s opinion about the necessity for bringing up in this discussion an intermediate steroid compound as the final active constituent when cortisone or compound F is given We all have observed marked improvement in inflammatory reactions particularly in the eye when a drop of cortisone is administered locally Doesn t this suggest that compound E or cortisone itself may be an effective local agent? It does not of course rule out more effective intermediary substances

Bauer The very fact that both compounds E and F are effective under these conditions raises many interesting questions which cannot be answered at this time It seems hard to avoid the conclusion that these hormones exert an anti inflammatory effect when dropped into an eye Whether this is due to the instilled hormone or an intermediate product we cannot say If the ocular lesion is a manifestation of a generalized disease like rheumatoid arthritis the local hormone therapy has no generalized effect when injected into a joint the hormone may exert both a local and systemic effect The very fact that ACTH cortisone and compound F exert a similar anti inflammatory effect in many other diseases is sufficient reason for us to speak of this beneficial action as anti inflammatory rather than antirheumatic even in the case of rheumatic fever and rheumatoid arthritis The problems concerning the mode of action of these hormones in inflammatory disease are many but well substantiated factual explanations are conspicuous by their absence

Sprague May I make one other remark? I am afraid the Conference might close with the thought that compound F acetate administered

intramuscularly is without metabolic activity This is not true We have found that if compound F acetate is given intramuscularly for a longer period of time than Dr Conn gave it it does induce metabolic changes which are qualitatively similar to those induced by cortisone The changes are less pronounced than those induced by cortisone but persist longer after administration of the steroid is stopped, possibly due to more prolonged absorption of the hormone from the sites of injection

Bauer In the two patients with rheumatoid arthritis whom we treated with compound F acetate parenterally we too observed little or no metabolic effects yet the anti inflammatory effect was comparable to that noted with the same dose of cortisone

Long I am sure that we could profitably extend this discussion very much longer but I know a good many of you are anxious to catch a train or a plane I would like to assure the Macy Foundation that the next two meetings planned for this Conference group will be made good use of judging by this one May I thank the gentlemen who came as our guests and participated in the program When they read the book that Dr Ralli hopes to produce in six months time they will be reassured that we found their presence here not only enjoyable but very constructive

REFERENCES

1 MEANS J H The integrative action of the endocrine system *Ann Int Med* 34 *1311 (1951)*

2 WILKINS L *et al* Treatment of congenital adrenal hyperplasis with cortisone *J Clin Endocrinol* 11, *1 (1951)*

3 SELYE H *Stress* Montreal Acta Inc 1950

4 ——— *Annual Report on Stress* Montreal Acta Inc 1951

5 FRIEDMAN S M POLLEY J R and FRIEDMAN C L Effect of desoxycorticosterone acetate on blood pressure renal function and electrolyte pattern in intact rat *J Exper Med* 87, *329 (1948)*

6 SPRAGUE R G MASON H L and POWER M H Observations on the pathogenesis of Cushing s syndrome *Proc Am Diabetes A* 10 *35 (1950)*

7 PRIESTLY J T *et al* Subtotal adrenalectomy for Cushing s syndrome A preliminary report of 29 cases *Ann Surg* 134, *464 (1951)*

8 MANN P J G and QUASTEL J H Benzedrine (β phenyl isopropylamine) and brain metabolism [illegible] 34 *41 (1940)* preliminary report Benzedrine a[illegible] *Nature London* 144, *943 (1949)*

9 WARD L E *et al* Clinical effects of [illegible] orally to patients with rheumatoid [illegible] *Mayo Clin* 26 *361 (1951)*

10 WHITE A *Symposium on Steroids in Ex*[illegible] *Practice* Philadelphia Blakiston 1951

intramuscularly is without metabolic activity This is not true We have found that if compound F acetate is given intramuscularly for a longer period of time than Dr Conn gave it it does induce metabolic changes which are qualitatively similar to those induced by cortisone The changes are less pronounced than those induced by cortisone but persist longer after administration of the steroid is stopped possibly due to more prolonged absorption of the hormone from the sites of injection

Bauer In the two patients with rheumatoid arthritis whom we treated with compound F acetate parenterally we too observed little or no metabolic effects yet the anti inflammatory effect was comparable to that noted with the same dose of cortisone

Long I am sure that we could profitably extend this discussion very much longer, but I know a good many of you are anxious to catch a train or a plane I would like to assure the Macy Foundation that the next two meetings planned for this Conference group will be made good use of judging by this one May I thank the gentlemen who came as our guests and participated in the program When they read the book that Dr Ralli hopes to produce in six months time they will be reassured that we found their presence here not only enjoyable but very constructive

REFERENCES

1 MEANS J H The integrative action of the endocrine system *Ann Int Med* 34, *1311 (1951)*

2 WILKINS L *et al* Treatment of congenital adrenal hyperplasia with cortisone *J Clin Endocrinol* 11, *1 (1951)*

3 SELYE H *Stress* Montreal Acta Inc 1950

4 ——— *Annual Report on Stress* Montreal Acta Inc 1951

5 FRIEDMAN S M POLLEY J R and FRIEDMAN C L Effect of desoxycorticosterone acetate on blood pressure renal function and electrolyte pattern in intact rat *J Exper Med* 87, *329 (1948)*

6 SPRAGUE R G MASON H L and POWER M H Observations on the pathogenesis of Cushing s syndrome *Proc Am Diabetes A* 10 *35 (1950)*

7 PRIESTLY J T *et al* Subtotal adrenalectomy for Cushing s syndrome A preliminary report of *29* cases *Ann Surg 134 464 (1951)*

8 MANN P J G and QUASTEL J H Benzedrine (β phenyl isopropylamine) and brain metabolism *Biochem J* 34, *414 (1940)* preliminary report Benzedrine and brain metabolism *Nature London* 144 *943 (1949)*

9 WARD L E *et al* Clinical effects of cortisone administered orally to patients with rheumatoid arthritis *Proc Staff Meet Mayo Clin* 26 *361 (1951)*

10 WHITE A *Symposium on Steroids in Experimental and Clinical Practice* Philadelphia Blakiston 1951